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REPRODUCTIVE HEALTH

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Review of
ESHRE 2016
Helsinki, Finland

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Welcome

Welcome to *EMJ Reproductive Health*, which presents the latest efforts being made to improve understanding and challenge the developing issues currently faced in the field. This edition provides a selection of interviews with our esteemed *EMJ Reproductive Health* Editorial Board. These offer an exciting chance to explore their successful careers alongside important insights into the future frontiers of reproductive health. There is also an extensive review of the 32nd Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE) from earlier this year.

ESHRE 2016 took place over 4 days in July and proved to be another successful and popular event for the society, covering the latest news and developments from human reproductive research and medicine. In this journal we have published a collection of abstract reviews from the authors themselves, whose insightful research was shared and discussed at ESHRE 2016. Also featured is a round-up of the most innovative and groundbreaking work presented at the congress. A freeze-all approach to *in vitro* fertilisation embryos increasing the rates of pregnancy and a challenge to the myth of the benefit experienced by short periods of rest after intrauterine insemination are among the stories recounted.

The peer-reviewed articles chosen for the journal this year include a case report from Farrakh et al. on the successful outcome of a perimortem caesarean section of an obese patient following cardiac arrest during labour. The report points to the clear value of performing such a procedure and the authors recommend its incorporation into the training of all obstetric staff. Govindan's literature review of 79 cases of Lipschütz ulcers draws important attention to the issue of misdiagnosis of these painful genital ulcers. It also points to the need for further research towards understanding the currently unknown aetiology of this challenging condition. Alongside these, you will find many more articles which cover a range of aspects of reproductive health.

Please enjoy this issue, which we have filled with informative and engaging content that we hope can be appreciated by every reader as well as being useful in practice. Thank you for reading and we look forward to bringing you another edition of *EMJ Reproductive Health* next year.

Spencer Gore

Director, European Medical Journal

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Front cover and contents photograph: Helsinki, Finland, home of ESHRE 2016.

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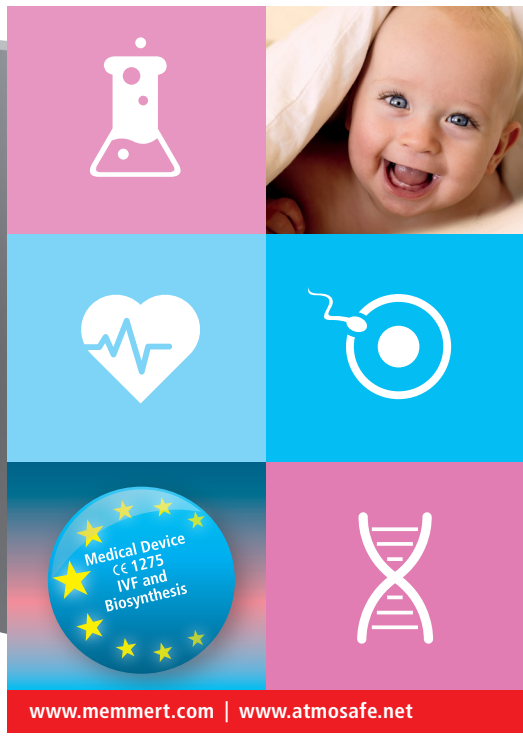

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Foreword

Prof Joep Geraedts

Emeritus Professor and Former Head of the Department of Genetics and Cell Biology, Maastricht University, Maastricht, Netherlands.

Dear Colleagues,

Welcome to this edition of *EMJ Reproductive Health*, in which a variety of interesting papers are presented.

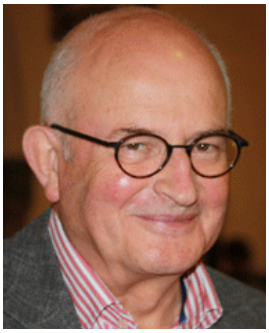
In a literature review Balaji Govindan reports on the causative organism of Lipschütz ulcers, which was unknown in the majority of patients (63%), while Epstein–Barr virus was the most frequently recognised micro-organism (16%). Sitruk-Ware et al. report on the progesterone vaginal ring which has been shown to be effective as a contraceptive in breastfeeding women who need a better method of spacing pregnancies. In an interesting case report, Saadia Farrakh et al. describes a sudden cardiac arrest in labour; the patient had a body mass index of 46 and by using perimortem caesarean section, a successful outcome was obtained.

Mariano Mascarenhas and Keerthi Gnanaprabha deal with the occurrence of monozygotic pregnancies, the risk of which is increased from 0.4% of natural conceptions to around 0.9–2.24% of pregnancies following assisted reproductive therapy.

This reminds me of two very interesting presentations regarding twin research at this year's Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE) in Helsinki, Finland. Bruno Reversade explained that he had characterised a hitherto unknown hormone ELABELA (ELA). This was done through genetic studies in a large kindred with familial monozygotic twinning. ELA is secreted by human embryonic stem cells and preimplantation embryos, where it orchestrates pluripotency and possibly the birth of monozygotic twins. At the same meeting, Dorret Boomsma reported on the findings of the Twinning GWAS Consortium to characterise the genetic basis of spontaneous dizygotic twinning. She reported that sequence variation at the *FSHB* and *SMAD3* loci increases the odds of dizygotic twinning and showed that variants in these two genes are significantly associated with a broad range of fertility and reproductive traits in women including age at menarche, age at menopause, age at first and last child, and lifetime parity. You can read more about the research that was presented at the congress in the abstract reviews, where some of the presentations have been summarised by the presenters themselves.

I hope you will enjoy reading this edition of *EMJ Reproductive Health* and find the coverage of this year's ESHRE congress interesting and informative.

Kind regards,



Joep Geraedts

Emeritus Professor and Former Head of the Department of Genetics and Cell Biology, Maastricht University, Maastricht, Netherlands.

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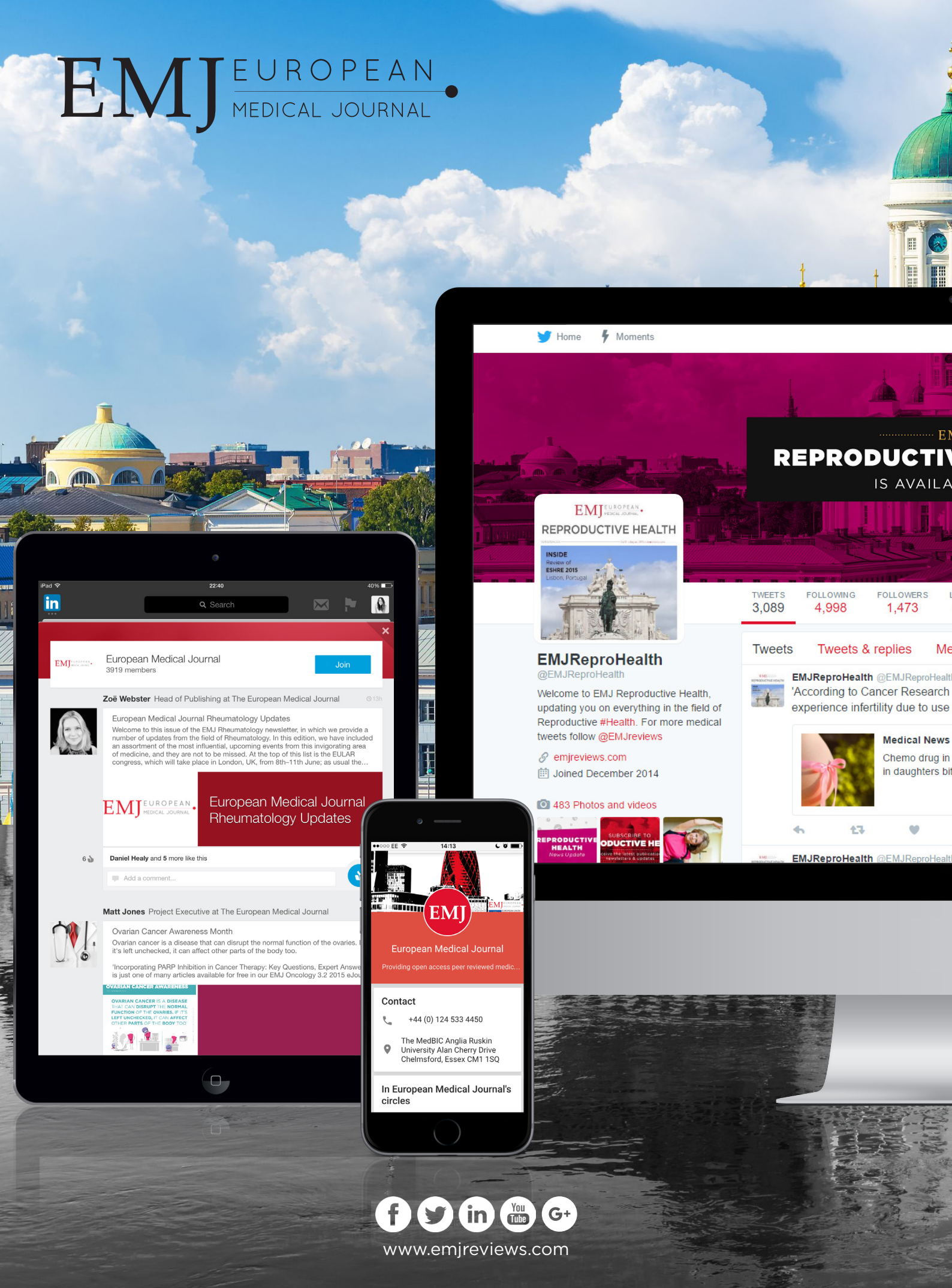

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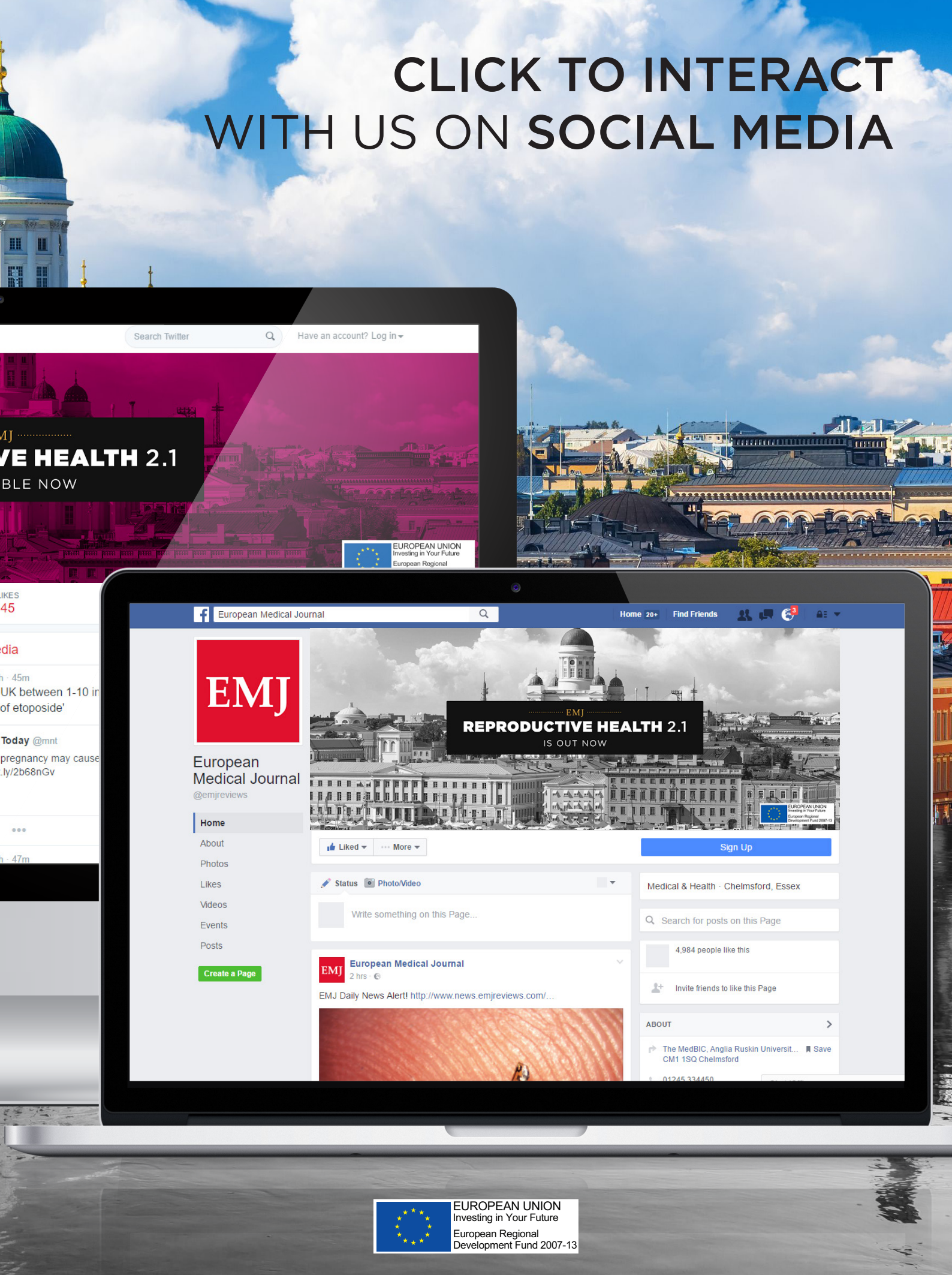

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ESHRE ANNUAL CONGRESS 2016

MESSUKESKUS EXPO AND CONVENTION CENTRE,
HELSINKI, FINLAND
3RD–6TH JULY 2016

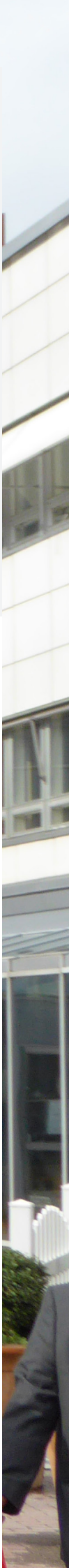
Welcome to the *European Medical Journal* review of the 32nd Annual Meeting of the European Society of Human Reproduction and Embryology Congress

The 32nd Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE) was held in Helsinki, Finland, a city with a reputation for award-winning design and a warm and welcoming culture. In 2000, it was one of nine chosen to be a European City of Culture. Finland successfully won the Eurovision Song Contest in 2006 and Helsinki hosted the following year, and in 2012 it was chosen as the World Design Capital. The city continues to be a central point of attraction for travellers across the world and for 4 days in July earlier this year, it was host to ESHRE 2016.

The ESHRE continues to grow as a society and its annual meeting draws thousands of participants every year. The society boasts over 6,000 members spread over 115 countries, making up a membership spanning across more than half of all the countries in the world. ESHRE 2016 also recorded impressive numbers this year with 1,764 abstracts received and almost 8,000 people registering to attend.

In other news at ESHRE 2016, six research authors were awarded €2,000 in recognition of their presentations. One author also received an educational travel grant to present their research again at the Fertility Society of Australia (FSA) Annual Conference 2016, taking place in Perth, Australia, from the 4th–7th September. The Basic Science Award for Oral Presentation went to Evelyn Telfer (UK). The research explored the effect of the chemotherapeutic regimen of adriamycin, bleomycin, vinblastine, and dacarbazine (used to treat lymphoma) on the number and development of ovarian follicles. It found an increase in the number of non-growing follicles in the ovarian cortex of adolescents and adults previously treated with ABVD for lymphoma. Kim Dreyer (Netherlands) was chosen for the Clinical Science Award for Oral Presentation, on account of her comparative research conducted into the efficacy of hysteroscopic proximal tubal occlusion by Essure® devices to treat hydrosalpinx in women undergoing *in vitro* fertilisation (IVF). The results showed that the method was inferior in comparison with laparoscopic salpingectomy in regards to ongoing pregnancy rates.

The Basic Science Award for Poster Presentation was awarded to Jessica Patel (UK). Following an analysis of over 1,900 kinetochores, her results showed that



sister kinetochores in human oocytes appear separated during the first meiotic division with each acting as separate attachment sites for microtubule fibres. These findings could provide an explanation for the high proportion of unstable attachments formed during meiotic division and indicate why oocytes are prone to aneuploidy.

The Clinical Science Award for Poster Presentation went to Talita Honorato (Netherlands). The research found that having a poor response twice in two consecutive cycles of IVF increased the risk of miscarriage, compared with having a poor response once or never before. Data was compiled from 5,427 women who underwent two cycles of treatment, with 773 ongoing pregnancies and 196 miscarriages applicable for analysis. The results were found to be independent of age. Poor response was defined as either three or less oocytes being produced during IVF treatment.

> “The ESHRE continues to grow as a society and its annual meeting draws thousands of participants every year.”

The Nurses Award went to Sara Somers (Belgium) for her oral presentation on the reproductive options for transgender men. Semi-structured interviews with female-to-male transgender people and their female partners in Belgium were conducted. The researchers found that couples felt their reproductive options were limited. In part, this may be due to Belgian law which states that transgender people seeking to change their legal sex must undergo physical gender adjustment, thereby affecting their ability to reproduce. However, a positive attitude was expressed by those aware of the possibility of generating gametes from stem cells, which would also allow both parents to have a genetic link to the child.

The ART Laboratory Award went to Vanessa Muyshond (Belgium). Her research found cell size and developmental state synchronicity in Day 2 and 3 embryos had an impact on blastocyst formation at Day 5. Non-stage-specific embryos made up 53.5% of the 1,817 fertilised oocytes analysed and they were found to have a reduced potential for further *in vitro* development. The findings provide researchers with a strong forecast for the developmental potential of embryos which could aid in their future selection during IVF.

Anna-Karina Hennginsen (Denmark) was the recipient of the Fertility Society of Australia (FSA) Exchange Award. Her research showed that the risk of major congenital malformations had remained stable in children conceived after assisted reproductive technologies (ART) in recent decades. A population-based cohort study was conducted that included 90,201 ART children using nationwide health registers from across Scandinavia. The researchers noted the existence of a well-known increased risk for ART children to these malformations. Their findings suggest the persistence of this risk despite progress made in clinical practice and research.

We now provide a collection of stories that highlight the latest research and important announcements from ESHRE 2016. This includes dispelling the myth of bed rest benefitting women following intrauterine insemination and pointing to the minimal impact of weight-loss on pregnancy rates among obese women undergoing fertility treatment. This review also offers an informative and engaging overview of the latest developments impacting reproductive healthcare and practice. We look forward to ESHRE 2017 in Geneva, Switzerland, and to hearing more about these research areas as well as what new progress has been made as a result.

Endometrial Scratch Procedure May Improve Chances of Pregnancy

ENDOMETRIAL scratching in women has been associated with an improved likelihood of live birth and ongoing pregnancy in some couples undergoing fertility treatment, according to a ESHRE press release dated 4th July 2016.

An assessment of randomised controlled trials that evaluated endometrial scratching in women planning to have intrauterine insemination or attempting to conceive spontaneously was undertaken by Cochrane collaborators. Endometrial scratching is a common result of routine biopsies of the womb lining thought to cause inflammation in the endometrium, inadvertently creating an environment much more receptive to embryo implantation in women undergoing *in vitro* fertilisation. The review shed light on whether endometrial scratching, either deliberate or accidental, is actually able to increase these chances.

> “the results must be handled with caution”

A total of 1,180 women, over eight trials, were included in the review. Endometrial scratching was compared with no intervention or a mock intervention. The primary outcomes measured were live birth and ongoing pregnancy, as well as pain from the intervention. In comparison with no procedure or the placebo procedure, endometrial scratching appeared to increase the chance of clinical pregnancy and live birth. The differences in outcome were found to be statistically significant with the chance of live birth following the procedure increasing from 9% over a set period of time to between 14% and 18% when compared with no intervention. Ms Sarah Lensen, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand, described endometrial scratching as: “a cheap and simple procedure” which can be conducted without analgesia during a short visit to a clinic. The review did not find any evidence that endometrial scratching has an effect on miscarriage, ectopic pregnancy, or multiple pregnancy.

4 days

Despite the positive outcomes of the review, "the results must be handled with caution" warned Ms Lensen, as the evidence collected from the randomised controlled trials was of a "very low-quality." Ms Lensen advised a caveat to these results because most of the trials included were associated with a serious risk of bias.

Birth Rates Improve Among Obese Women Who Lose Weight and Conceive Naturally

WEIGHT-LOSS in obese women who undergo fertility treatment has little impact on birth rate, but makes a significant difference in pregnancies following natural conception.

This is according to the results of a large randomised controlled trial that assessed the effect of lifestyle adjustment on fertility in obese women, later confirmed in a subsequent subanalysis of the data. These results have been reported in a ESHRE press release dated 4th July 2016. In the study, an intervention group of 290 obese women was assigned to a 6-month weight-loss programme which preceded 18 months of fertility treatment. In the control group, 287 obese women were assigned to fertility treatment within the same 24-month period. The mean weight loss was 4.4 kg in the intervention group and 1.1 kg in the control group. Birth rates for each group following this was 35% and 27%, respectively. When including data on the ongoing pregnancies conceived within the 24-month period, the difference in birth rates became even slighter (control: 53%, intervention: 58%).

> Our finding that lifestyle intervention in obese women more often leads to natural conception, specifically in anovulatory women, should be used in their counselling before fertility treatment and could reasonably be offered as first-line treatment for anovulation in obese women.

In comparison, the rate of ongoing pregnancies after natural conception was significantly higher for women in the weight-loss programme than those undergoing fertility treatment. The subanalysis of the data calculated rates of vaginal birth of a healthy singleton between the two groups and also according to six different subgroups. These subgroups were defined by age (over or under 36 years), cycle regularity (ovulatory or anovulatory), and body weight (above or below a body mass index [BMI] of 35 kg/m^2).

A significant beneficial effect from the weight-loss programme on birth rates was found for women who were anovulatory after natural conception. The results showed 28.6% (35/123) of the anovulatory women who naturally conceived achieved a healthy birth, in comparison with 11.4% (16/140) of anovulatory women who underwent fertility treatment.

Dr Anne van Oers, University Medical Centre, Groningen, Netherlands, who presented the results of the subanalysis study at ESHRE 2016, concluded: "Our finding that lifestyle intervention in obese women more often leads to natural conception, specifically in anovulatory women, should be used in their counselling before fertility treatment and could reasonably be offered as first-line treatment for anovulation in obese women."

Short Periods of Rest After Intrauterine Insemination Does Not Increase Pregnancy Rates

KEEPING female patients immobilised after intrauterine insemination (IUI) does not have a beneficial effect on pregnancy rate, despite the widely held belief that this is the case.

This is according to the results of a large randomised controlled trial which have been reported in a ESHRE press release dated 5th July 2016. The comparative study involved 479 patients with standard indications for IUI, such as unexplained or mild male factor infertility. The patients were assigned to either 15 minutes of immobilisation immediately after insemination or to immediate mobilisation after the treatment. For most patients the treatment course involved several cycles of IUI; the comparison between the two groups was then based on a total of 950 cycles of immobilisation and 984 cycles of mobilisation.

The results showed that the cumulative ongoing pregnancy rate per couple was not statistically significant when compared between the two groups. A pregnancy rate of 32.2% was measured after 15 minutes of immobilisation and a rate of 40.3% after immediate mobilisation. For researchers involved in the study, the results indicated no benefit from a brief period of rest after insemination. "In our opinion, immobilisation after IUI has no positive effect on pregnancy rates, and there is no reason why patients should stay immobilised after treatment," explained Dr Joukje van Rijswijk, Medical Researcher, Vrije Universiteit Medical Center, Amsterdam, Netherlands.

> "We also know from other studies that sperm cells can reach the fallopian tube 5 minutes after intravaginal insemination and that they can survive for several days in the womb. Why should bed rest affect that? There is no biological explanation for a positive effect of immobilisation."

The authors of the study acknowledge that their findings dispute the results of previous research which have led to a widespread acceptance of bed rest after IUI. For example, a small Dutch study published in 2009 concluded that 15 minutes of bed rest improved the pregnancy rate and this should be offered to all women treated with IUI.

"Our goal was to replicate the results. There is always a possibility that a positive outcome in studies is the result of chance. We also know from other studies that sperm cells can reach the fallopian tube 5 minutes after intravaginal insemination and that they can survive for several days in the womb. Why should bed rest affect that? There is no biological explanation for a positive effect of immobilisation," Dr van Rijswijk added.

Large Danish Study Reveals that Fertility Treatment Works

RESULTS of a large cohort study that analysed the health records of women in Denmark have indicated that three out of four women who begin fertility treatment will have a child within 5 years.

Researchers evaluated the health records of 19,884 women in Denmark who received fertility treatment between 2007 and 2010, tracking the number of live births after 2, 3, and 5 years. The results were reported in a ESHRE press release dated 6th July 2016, demonstrating that at 2-year follow-up, 57% of the women had had a baby as the result of fertility treatment and 14% conceived without treatment. Of those receiving fertility treatment, 46% conceived by first-line *in vitro* fertilisation (IVF). In comparison, the total rate delivered after intrauterine insemination (IUI) when used as the first-line treatment was 34%.

The extensive registry records available in Denmark, which link all fertility treatments with all live births, enabled the large scale study. This also provided robust results that could offer real-life prognosis. "We are now able to provide couples with a reliable, comprehensible, age-stratified, long-term prognosis at [the] start of treatment," explained lead author of the study Dr Sara Malchau, Copenhagen University Hospital, Hvidovre, Denmark.

> We are now able to provide couples with a reliable, comprehensible, age-stratified, long-term prognosis at [the] start of treatment.

The analysis showed that age was the greatest determinant of success in those who received fertility treatment. Out of the 5,165 women available for follow-up at 5 years, the total birth rates were 80% for those aged <35 years, 60.5% for those aged 35–40 years, and 26% for those aged >40 years. The researchers noted that the information based on the age of the patient also informed the chances of natural conception. After 5 years from IUI as first treatment, 18% of women <35 years old had given birth after spontaneous conception, in contrast with only 8% of women >35 years starting treatment with IVF. The researchers concluded that this study will allow for a long-term prognosis to be offered to couples starting fertility treatment.

Levels of Mitochondrial DNA Found to be Accurate Marker for Embryo Viability

A NEW approach to embryo selection offers an accurate prediction of embryo viability and insight into why it fails to produce pregnancy when found without chromosomal anomalies.

The approach is based on a quantification of mitochondrial DNA (mtDNA) found in the outermost layer of cells in a 5-day-old embryo, also known as a blastocyst. In combination with chromosome analysis that screens abnormality or aneuploidy, it is a method that can be used to improve *in vitro* fertilisation (IVF). These abnormalities in the number of chromosomes in embryo cells are considered the main reason for miscarriage and implantation failure. However, even when an embryo has a normal number of chromosomes its implantation often results in a failure to produce pregnancy roughly one-third of the time.

> The results confirm that embryos with elevated mtDNA rarely implant and support the use of mitochondrial quantification as a marker of embryo viability.

Reported in a ESHRE press release dated 4th July 2016, a study of 280 blastocysts with a normal number of chromosomes was used to evaluate the levels of mtDNA as an accurate marker of embryo viability. The mtDNA levels in the blastocysts were not known prior to being transferred to patients undergoing IVF, so the study results relied on a comparison of IVF outcome and mtDNA levels. Of the 111 single blastocyst transfer outcomes known to the researchers, 78 led to ongoing pregnancies and each of those had levels of mtDNA considered normal. There were 33 blastocysts that failed to implant and 8 of these had unusually high levels

of mtDNA. The study showed that 78/103 blastocysts had normal levels of mtDNA, good morphology, and a normal number of chromosomes, resulting in ongoing pregnancy. The results showed 0/8 blastocysts achieved pregnancy with unusually high levels of mtDNA but were otherwise normal.

Dr Elpida Fragouli, Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, UK, stated that: "The results confirm that embryos with elevated mtDNA rarely implant and support the use of mitochondrial quantification as a marker of embryo viability." Dr Fragouli went on to emphasise that embryos must first be screened for aneuploidy and the mtDNA test is applicable only to embryos with a normal number of chromosomes.

'Freeze-all' Approach to IVF Embryos Increases Rates of Pregnancy

THE APPROACH of freezing all embryos generated for *in vitro* fertilisation (IVF) for later transfer in to the uterus has been associated with higher success rates of pregnancy, particularly in women aged >35 years old.

8,000 people registering to attend

This is according to the results of a study reported in a ESHRE press release dated 6th July 2016. In the study, 16,206 IVF treatment cycles were examined retrospectively in 12 fertility centres in the USA, including 2,100 freeze-all cycles, between 2009 and 2015. The researchers used logistic regression to compare freeze-all with matched fresh control cycles to measure the odds of ongoing pregnancy.

The analysis showed that the treatment approach of freezing all IVF embryos for transfer in a subsequent cycle was significantly associated with improved ongoing pregnancy rates in patients in those over 35 years old. The rates were measured at 46% in the freeze-all approach and 33% in the fresh cycles. The researchers also found that elevated levels of progesterone prior to egg retrieval benefitted patients, and this was considered to be evident regardless of age. Among those with the elevated levels, a birth rate of 47% among freeze-all cycle patients and 38% among fresh cycle patients was recorded in those younger than 35 years. For those above 35 years of age, the rates were 45% and 30%, respectively.

> "...we cannot yet conclude that this is an effective strategy for any group of patients."

"There are several reasons clinics do freeze-all cycles," explained Dr Eric Widra, Medical Director, Shady Grove Fertility, Washington DC, USA. He continued: "These include patients at high risk for ovarian hyperstimulation syndrome, patients having preimplantation genetic diagnosis prior to embryo transfer, and importantly, those patients who have a premature rise in the concentration of progesterone hormone prior to egg retrieval. Several studies have shown that this rise in progesterone is associated with a lower pregnancy rate after fresh embryo transfer."

Dr Widra also acknowledged that the freeze-all approach has not been widely adopted and further research is needed before definitive conclusions can be drawn on its efficacy. He said: "The evidence of this study is intriguing, but without prospective randomisation, we cannot yet conclude that this is an effective strategy for any group of patients."

Children Adjust Equally Well in Solo Mother Families

SIGNIFICANT differences in child adjustment do not exist between families formed by single women using donor sperm and heterosexual two-parent families with at least one donor-conceived child, suggests a study performed at the Centre for Family Research at the University of Cambridge, Cambridge, UK, the findings of which have been reported in a ESHRE press release dated 4th July 2016.

The results suggest that children aged between 4 and 9 years old, who belong to a family type where the father is absent, continue to be generally well-adjusted when actively aware of the absence. "Indeed, at the age at which children begin to understand their family circumstances, they continue to function well," explained an author of the study Dr Sophie Zadeh, Centre for Family Research, University of Cambridge, Cambridge, UK.

The study involved 51 solo mother families using a standardised questionnaire and semi-structured interviews; 47 children within these families agreed to be interviewed. Questionnaires were also administered to mothers from 52 heterosexual two-parent families with at least one donor-conceived child. No significant difference in measurements of child adjustment were found when compared between the two family types.

> "In general, our findings seem to suggest that what matters most for children's outcomes in solo mother families is not the absence of a father, nor donor conception, but the quality of parenting, and positive parent–child relationships."

Conversations about the absence of a father were reported to be a prominent feature in family life by solo mothers. When asked about changing their family circumstances, 38% of the children expressed a desire for trivial changes and 51% wanted no change. Of those speaking about their enjoyment of school, 19% reported a high level of enjoyment and 40% reported a very high level. All of the children reported having at least one friend, and 51% could name five or more friends.

"In general, our findings seem to suggest that what matters most for children's outcomes in solo mother families is not the absence of a father, nor donor conception, but the quality of parenting, and positive parent–child relationships. These findings therefore echo much of what we already know about the determinants of children's psychological adjustment in other family types," Dr Zadeh concluded.

Doubt About the Benefit of Human Growth Hormone for IVF Patients

RESEARCHERS have cast doubt over the use of human growth hormones for improving live birth rates for women undergoing *in vitro* fertilisation (IVF).

The results of a randomised placebo-controlled trial were reported in a ESHRE press release dated 4th July 2016. It showed that live birth rates were no better in poor-responding patients who were given human growth hormone as a supplement to IVF than in patients given a placebo. Poor-responders were defined as women who had undergone a previous IVF cycle and produced no more than five eggs after ovarian stimulation.

> "some studies, many of them small, do reflect a trend towards improved clinical pregnancy outcome."

Prof Robert Norman, Robinson Research Institute, University of Adelaide, Adelaide, Australia, and colleagues, expressed concern for the applicability of findings in previous studies in regards to significant clinical heterogeneity and uncertain safety outcomes, commenting that the studies "do not address the most important clinical outcome our patients require, that of delivering a live healthy infant." However, he went on to say that, "some studies, many of them small, do reflect a trend towards improved clinical pregnancy outcome."

The results of this study showed a clinical pregnancy rate of 14% in the group receiving growth hormone and a rate of 11% in the placebo group. There were equal comparisons between the two groups in the number of eggs collected, quality of embryos, and duration of treatment. These results implicate a continuing lack of evidence as to whether a benefit is gained from human growth hormone when used by poor-responders as a supplement to IVF treatment. The researchers noted that this can only be determined by an extremely large randomised trial. A concern with the previous trials was that they were underpowered and this was also an issue that faced the researchers in their own study. After 4 years, only 136 women under the age of 41 years had been recruited. The low number was partly attributed to many prospective recruits buying growth hormone outside the trial.

The team point to the difficulties faced in treating patients who are poor-responders to IVF treatment and that although many strategies have been tried, no strong evidence exists as to whether they improve live birth rates; including that of human growth hormone. This factor, along with the cost, means that such treatment may be not be as beneficial as once thought.

1,764 abstracts received

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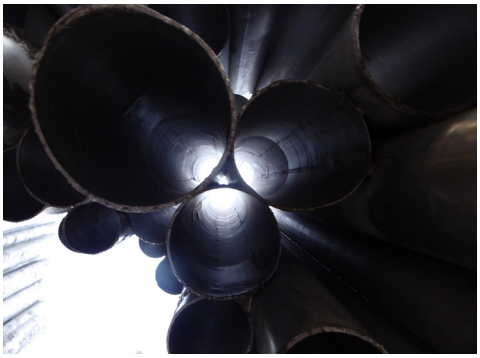
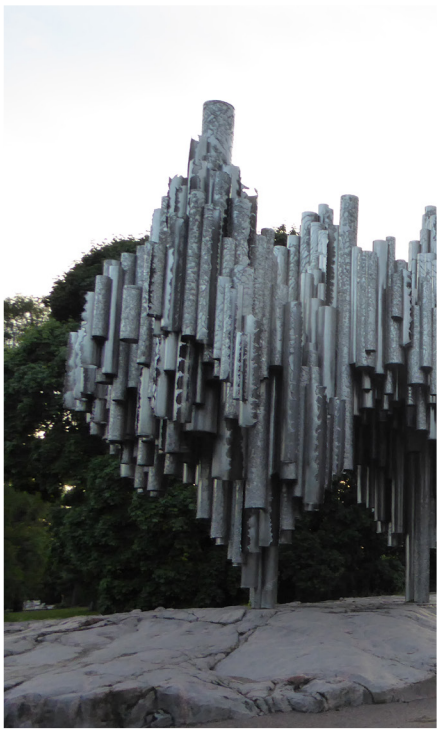
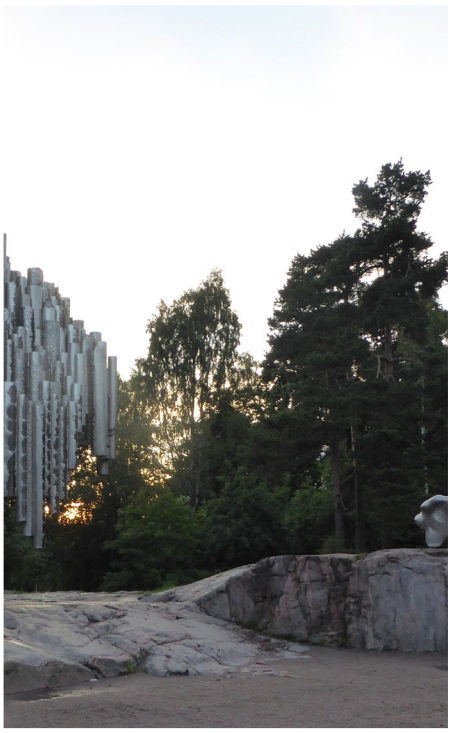
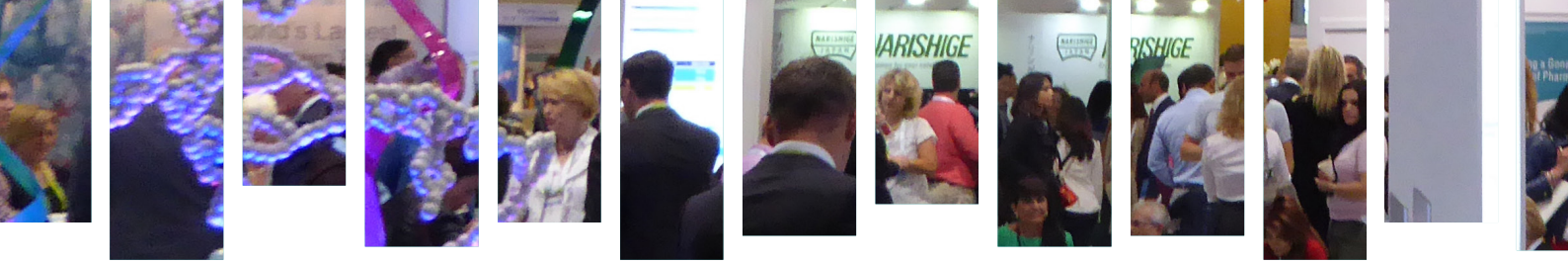








Liselotte Mettler

Emeritus Professor, Department of Obstetrics and Gynaecology, University Hospitals Schleswig-Holstein, Kiel; Honorary Patron, Kiel School of Gynaecological Endoscopy & Reproductive Medicine; General Manager of Gyne Consulting Kiel, Kiel, Germany; Lecturer and Visiting Professor, German Medical Center, Dubai Healthcare City, Dubai, UAE.

Q: What motivated you to begin working in the field of reproductive health and to focus your research on endocrinology and gynaecology?

A: The rapid progress in scientific and clinical findings over the last 40 years, including assisted reproductive technology and the development of endoscopic gynaecological surgery.

Q: What specific areas of research are you currently exploring and what developments do you hope to achieve from doing so?

A: Fertilisation and its trigger. The underlying molecular genetics.

Q: To what extent has the development of assisted reproductive technology improved since you first began working in this area?

A: From simple *in vitro* fertilisation to a better understanding of molecular genetic connections.

Q: Do you anticipate the introduction of any new reproductive technologies or innovations upon previous technologies that will be used beyond a therapeutic function in the coming years?

A: I definitely do in the field of transplantation, genetic diagnosis, and genetic treatment.

Q: Please could you tell us about your time spent in the Dubai Healthcare City, a free economic zone for healthcare, and your work with the German Medical Centre situated there?

A: Since 2007 I have had the pleasure of occasionally working in that location and since 2010 I have regularly advised and consulted doctors and patients in the field of gynaecologic endoscopic surgery.

Q: Can you speak on the impact that the pioneering endoscopic surgeon, Kurt Semm, has had on your career?

A: I have worked 25 years with Kurt Semm and developed many endoscopic surgical procedures under his guidance and with him. Our co-operation influenced my career greatly.

Q: Your new book 'Hysterectomy: A Practical Guide' is due to be published later this year. Do you expect any significant advances in the efficacy of hysterectomy procedures to emerge within the next few years?

A: Endoscopic hysterectomy already is the technology of choice. I think with better imaging connections it will be possible to do these surgeries robotically with previous planning alongside radiologists.

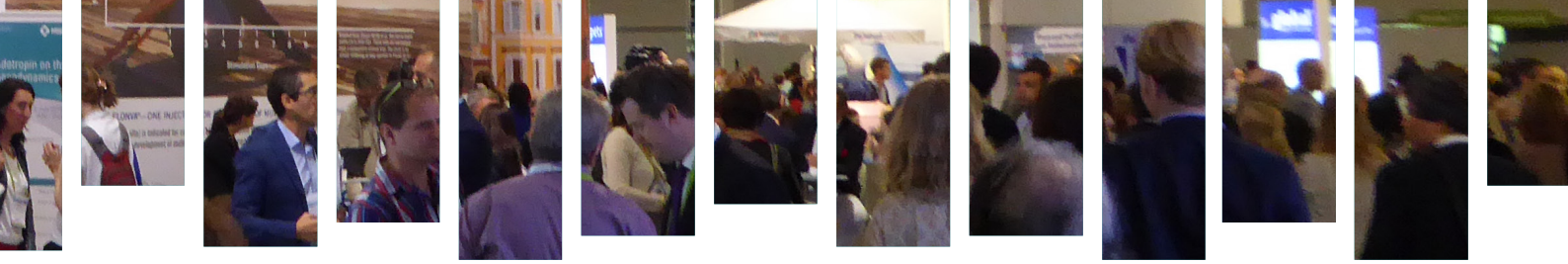
Q: How do you feel the standard of research and clinical practice in reproductive medicine across Europe compares to other parts of world?

A: I would say that we are the leaders in reproductive medicine.

Q: What has been the proudest achievement of your career to date? What specific goals do you hope to achieve in future work?

A: Our first test tube baby was in Kiel in 1981. In the field of endoscopy my first endoscopic treatments of ectopic pregnancies were in 1972. I hope to be involved in the programming of an entirely robotic surgical procedure under imaging control.

> "The field of reproductive health and endoscopic surgery is fascinating..."



Q: What advice do you have for medical students who are thinking of beginning a career in reproductive health? What, in your opinion, is the most exciting aspect of working in this field?

A: The field of reproductive health and endoscopic surgery is fascinating and gives medical students a clear career direction.

Joep Geraedts

Emeritus Professor and Former Head of the Department of Genetics and Cell Biology, Maastricht University, Maastricht, Netherlands.

Q: When did you first take an interest in genetics and reproductive science? Who or what inspired you to pursue this career path?

A: In 1969, Lee Douglas taught me how to cross Drosophila when I was studying for my master's degree, and in 1972, Peter Pearson, my supervisor at Leiden University during my PhD, encouraged me to look at the interphase chromosomes in sperm cells. That was the beginning of my still continuing interest in many different topics at the crossroads of genetics and reproduction.

Q: You have worked in the field for almost 45 years, what do you think has been the most significant research breakthrough in the sphere of reproductive health to date?

A: The most impressive breakthrough by far has been the introduction of *in vitro* fertilisation (IVF). The real objective measure of its significance is that more than 5 million children have been born already making use of IVF, but also that Bob Edwards was awarded the Nobel prize for it in 2010. He deserved it without any doubt but it was disappointing that it took 32 years after the birth of Louise Brown before this happened.

Q: How would you describe the rate of progress in current reproductive science? How do the methods used today compare with when you started out as a researcher?

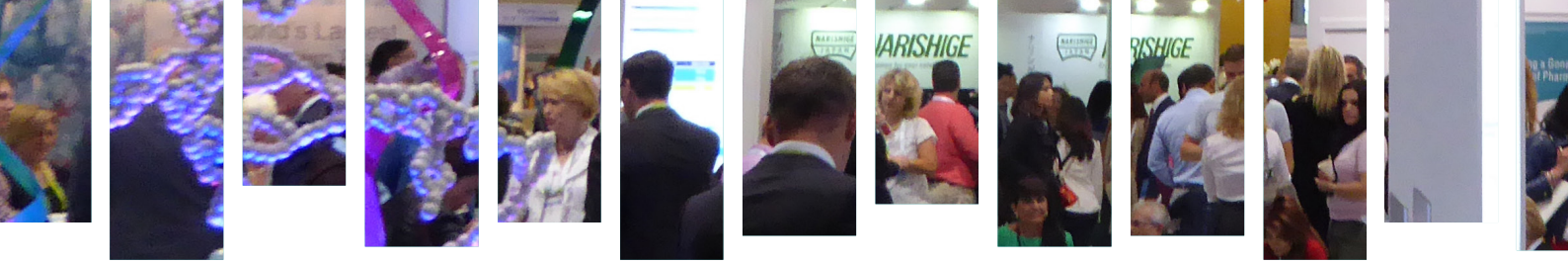
A: In terms of both genetics and assisted reproduction we now live in a completely different world. I do not think that any other discipline has seen so much progress in such a short time period. As far as genetics is concerned I can give you many examples, but perhaps it is best to mention that we are now able to sequence the complete genome of a single cell. In assisted reproduction, I would say first intracytoplasmic sperm injections and later vitrification have contributed enormously to this success.

Q: In previous interviews you have mentioned that there are ethical and legal challenges to solve before genetic research can advance further. Has there been any progress in this area in recent years, and do you think there will ever be a way to fully resolve these issues?

A: There are too many countries that have strict laws with respect to research on preimplantation embryos. In my opinion it should be possible to create embryos for research. However, only a few countries allow that. It is interesting to see that, for instance, in our country (Netherlands), embryos are very much protected when it concerns researching embryos in the lab, whereas when it comes to morning-after pills, they are sold without difficulty in drug stores.

Q: Where do you see our understanding of genetics developing in the next 20 years if these challenges are worked out? What changes would you like to see in the field?

A: There are many areas where there is room for development: genetic screening, personalised genetics, and epigenetics, especially in relation to the transgenerational effects of it. However,



before genetics has a place in the everyday life of patients and doctors, the latter should be educated and trained further in order to counsel their patients properly.

Q: What has been the proudest achievement of your research and career?

A: In 1986 Chris Höweler wrote a thesis in which he proved the existence of Anticipation, defined as such: 'The signs and symptoms of some genetic conditions tend to become more severe and appear at an earlier age as the disorder is passed from one generation to the next'. Many clinicians have observed this since the first half of the last century. However, in genetics textbooks its existence was denied, as it resulted from bias of ascertainment. Since almost nobody would read his thesis, I tried to convince Chris that he should publish his findings in a scientific paper. For about 3 years I helped him to write it and in 1989, the paper 'Anticipation in myotonic dystrophy: Fact or fiction?' was published in a journal. At the beginning of the new century Peter Harper published a book with 47 classic papers, which he called Landmarks in Medical Genetics. Our paper on Anticipation was one of these and the only one from our country.

Q: How do you think genetic research differs across Europe and internationally and what are the effects of these differences?

A: It is hard for me to have an opinion on genetic research in general since this involves all organisms and all sorts of research. As far as human reproductive research is concerned you can see that for years the same countries are in the lead: UK, Belgium, Spain, and a few others. This must have to do with the fact that the laws are liberal. Furthermore, a relatively large number of patients are treated in these countries which provides plenty of opportunities to come into contact with patient material which is indispensable.

Q: You have written and contributed to a huge number of books and articles over your career. What has been your favourite subject to write about and is there a particular area of your field which you feel deserves more coverage or attention?

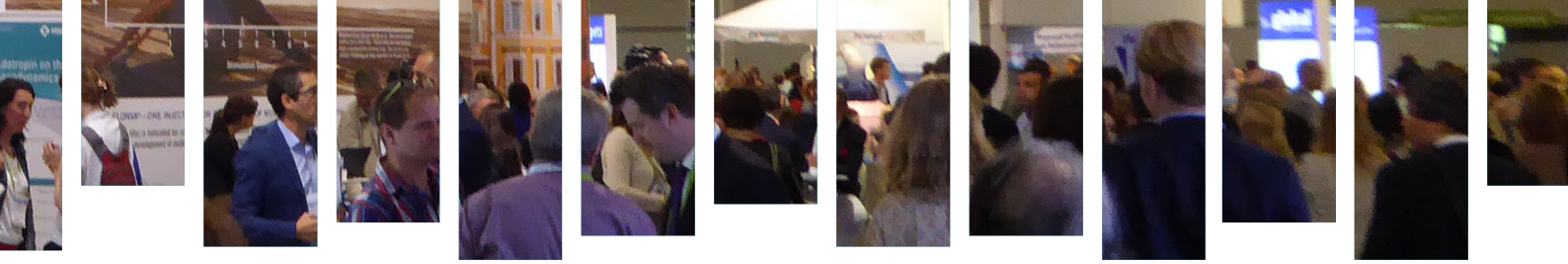
A: I always felt attracted or even obliged to explain to the general public the developments that were going on in genetics and reproduction, such as cloning, preimplantation genetic diagnosis, etc. in order to create support. At the moment I am more interested in evolution. In fact, I not only make that more known to the public through writing, but also in a newly established museum of which I am the chairman of the board. We started 2 years ago to bring together family history, DNA, human evolution, etc. and at the moment we are still building.

Q: You are the founder of the Genetics Retreat, an annual meeting for PhD students and their supervisors. How do events like this one and ESHRE benefit researchers, particularly those who are new to the field, and how do they influence the research being undertaken?

A: I not only founded the Genetics Retreat 26 years ago but ever since that time I have organised a new one each year. In the environment of a 900-year-old monastery, PhD students can gain proficiency in discussing their ongoing research and develop their presentation skills in an informal but professional setting. Furthermore, over the years many experienced speakers have delivered a keynote lecture after dinner which was thereafter discussed until midnight at the bar. I think for youngsters it is important to create an informal atmosphere to have a successful meeting.

Q: What advice would you give to young medical students looking to specialise in genetics?

A: Be prepared to move. Try to gain experience in as many different disciplines and places as possible at the start of your career. It is not only that you learn different methods or techniques but you also learn to judge different people with their different cultural and other backgrounds. I think this is important since research is becoming more and more global. After such a start you can stay in one place to try to specialise in your favourite discipline.



Eduard Ruiz Castañé

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Q: You have spent a large portion of your career studying infertility, especially male infertility. How has our understanding of the causes of infertility and our treatment options for it developed over the past few years?

A: Male infertility is a complex disease with heterogeneous phenotypic representation and in at least 15% of cases, this condition is related to known genetic disorders, including both chromosomal and single-gene alterations. In about 30–40% of primary testicular failure the aetiology remains unknown and genetic anomalies remain unidentified. Well-established genetic causes of male infertility are limited to Y chromosome microdelections and Klinefelter's syndrome, together accounting for 10–20% of cases of severe spermatogenic failure. Significant recent advances in micro and next-generation sequencing technologies have enabled the application of whole genome approaches to the study of male infertility.

Q: In your opinion, what proportion of infertility cases can be attributed to genetics or lifestyle?

A: Recently, the pivotal role that lifestyle factors play in the development of male infertility has generated a considerable amount of interest. Lifestyle factors are the modifiable habits and ways of life that can greatly influence overall health and wellbeing, including fertility. Many lifestyle factors such as the age at which to start a family, nutrition, weight, exercise, psychological stress, environmental and occupational exposures, and others, can have a substantial effect on fertility. Lifestyle factors such as cigarette smoking, drug use, alcohol, and caffeine consumption have a negative influence on fertility.

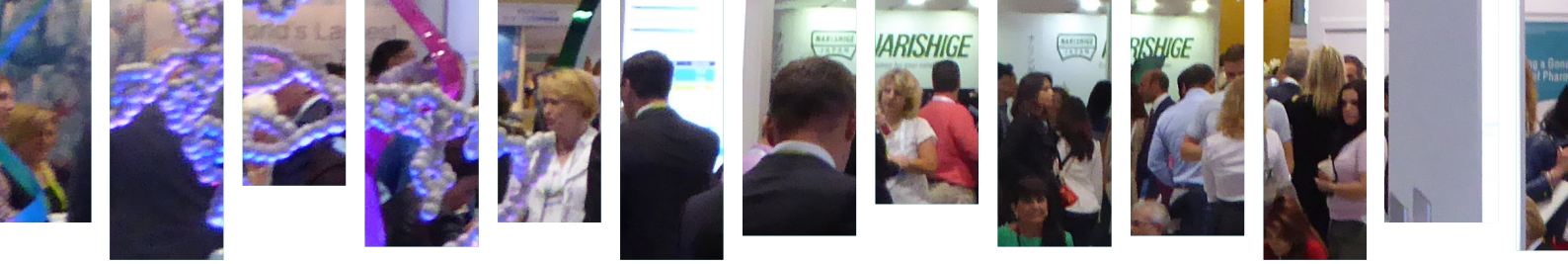
In addition to this, the novel approaches in genetic factors give a more extensive picture of the aetiopathogenesis of idiopathic male infertility and help to understanding the complex world of gene and environment interaction, and epigenetics.

Q: How do meetings such as the ESHRE Congress contribute to the advancement of research in these areas?

A: These meetings promote the understanding of reproductive biology and embryology. They facilitate research for all members and in select sexes, special interest groups such as andrology, reproductive genetics, ethics and law, and paramedics, while informing us of medical education activities.

Q: What more can patients be doing to take care of their reproductive health or fertility? Are there any very basic lifestyle changes that could dramatically enhance a person's reproductive health in your opinion?

A: Lifestyle factors may also contribute to the observed adverse trends in male reproductive health. During the past 50 years, huge changes in Western lifestyle have occurred; for example, obesity is reaching epidemic proportions worldwide. The prevalence of smokers has also increased and then more recently declined in many Western countries. Several studies among men from the general population or infertile men have shown that male obesity is associated with reduced semen quality. Smoking has also been



found to impair semen quality. A recent study among men from the general population found a dose-response relationship between smoking and sperm motility and total sperm count.

Interestingly, maternal smoking during pregnancy has a quite pronounced negative impact on semen quality among the offspring, indicating that prenatal exposures are also important. Maternal smoking in pregnancy has also been shown in some (but not all) studies to increase the risk of hypospadias and cryptorchidism in male offspring. On the other hand, a meta-analysis has shown that maternal smoking during pregnancy is not associated with increased risk of testicular germ cell carcinoma in sons. Nevertheless, the considerable increase in smoking prevalence among young women in most European countries in recent years can only exacerbate the incidence of male reproductive problems as some of these women will continue to smoke during pregnancy.

Q: What advice would you give to couples suffering from fertility problems? What are the best options for them to consider?

A: There are solutions to all problems, whether it is male or female infertility. Improved lifestyle and contact with an andrologist and also a gynaecologist to assess the problem together, rule out any recoverable aetiology, and in borderline cases, initiate a process of assisted reproduction.

Q: How do approaches to reproductive health differ across Europe? Do you think anything could be learned from the Spanish approach?

A: Rather than purely a Spanish approach, there are currently recognised centres as well as an increased effort from various associations and medical societies to unify criteria with consensus meetings, as well as the publication of European guidelines. We work on the line to assess both partners in the infertile couple with exhaustive studies of all fields to establish, if possible, the type of infertility and then be able to provide specific treatment for each case.

Q: Can you tell us about your current research interests as well as your professional goals for 2016?

A:
- The decline in semen quality
- Endocrine disrupting chemicals
- Testicular dysgenesis syndrome
- Preservation of fertility in oncologic patients
- Lifestyle factors and the influence in fertility
- New genetic factors

Q: What do you think is the most pressing issue for men's reproductive health at the moment?

A: Genetic and lifestyle factors are the most pressing issues for men's reproductive health. We have improved the results in congenital malformations, immunity, germ tumours, hormonal disorders, and sexually transmitted infections but there are new areas of development that hit us and represent new challenges.

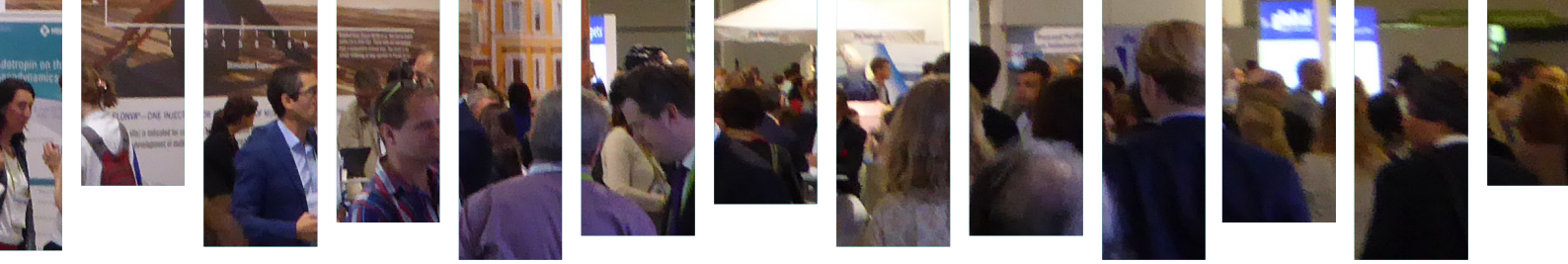
Q: Do you believe that men's health problems have worsened, or are left untreated due to a lack of awareness, or even embarrassment regarding problems or symptoms?

A: Yes. It is difficult to educate both professionals and patients to invest in research and prevention of many diseases which results in a better sexual and reproductive health. We do not have to wait for symptoms or problems. We have to act preventively and consider the difficulty before it appears.

Q: What changes would you like to see in your field during the next few years?

A: I expect an improvement in research with greater provision of public and private media. I hope more training for both doctors and patients becomes available. If there is an increased awareness, we will get better results.

> "Interestingly, maternal smoking during pregnancy has a quite pronounced negative impact on semen quality among the offspring indicating that prenatal exposures are also important."



Q: What do you think have been the most significant advancements in reproductive medicine since you began your career?

A: My professional trajectory is long and I have lived many steps, but globally I think that the advances in reproductive medicine have been spectacular.

Diagnostics:

- Genetic studies
- Iconographics (RMC, ultrasounds)

Therapeutics:

- Assisted reproduction
- General practice, *in vitro* fertilisation, intracytoplasmic sperm injection

Antonio Simone Laganà

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Q: What inspired you to specialise in the field of reproductive health? Was there a particular person or event that swayed your decision?

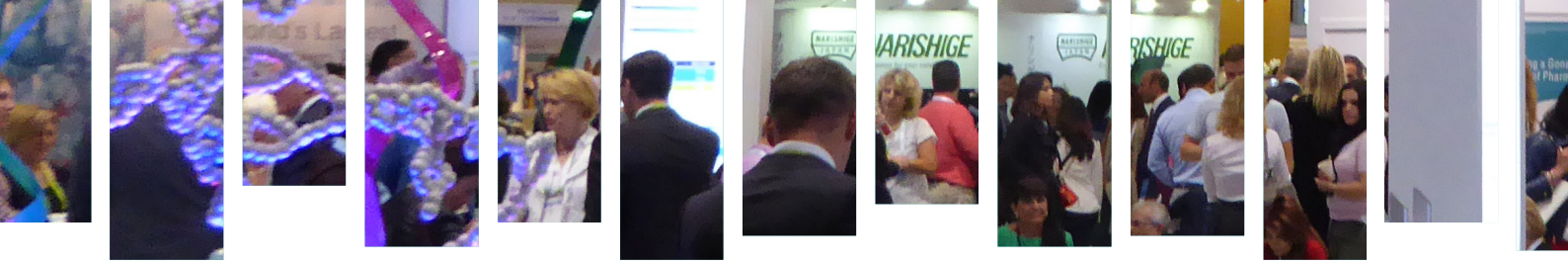
A: I decided to dedicate my professional career to reproductive health because I think that it represents one of the main clinical and research fields of all medicine. The specialist focussed on reproductive health has a complete approach to ensure women's health using several diagnostic as well as therapeutic techniques. Furthermore, the obstetrics and gynaecology specialist has to unravel numerous different research questions, which are still far from elucidated.

Q: You have worked with a vast number of societies and organisations throughout your career. What projects are you most proud to have worked on or be working on currently?

A: I have the opportunity to be a member of many important international societies, such as the Society for Reproductive Investigation (SRI), ESHRE, International Society of Gynecological Endocrinology (ISGE), International Society of Ultrasound in Obstetrics and Gynecology (ISUOG), and others. In particular, I am very proud to be Junior Deputy of the Special Interest Group Endometriosis and Endometrial disorders of the ESHRE, which combines a basic and a clinical focus on research and training related to endometriosis and endometrial disorders (e.g. endometrial pathophysiology, adenomyosis, fibroids, abnormal uterine bleeding, pelvic pain syndrome).

Q: You have studied endometriosis for a lot of your career. How has our understanding of this condition developed since you began working in this field, and how are treatments improving?

A: If I look back to when I began to study the aetiology of endometriosis, many things have radically changed. Considering the cutting-edge research of the last decade, we currently have a better view of how the disease develops and about the close connection between immune response, hormonal homeostasis, and the genetic and epigenetic background. Nevertheless, new and strong efforts are needed to unravel the pathogenesis of the disease and to offer patients the best evidence-based treatments, possibly tailored to each different individual.



Q: How do congresses such as ESHRE help with the advancement of our understanding of reproductive disorders and fertility? Are there any presentations this year that you are particularly looking forward to?

A: In my honest opinion, international congresses such as ESHRE are the best moments for the advancement of our understanding of reproductive disorders and fertility, since they represent a platform to connect experts from different subspecialties thus allowing a close collaboration on the most updated research topics.

Q: What advice would you give to young doctors who are interested in specialising in the field of reproductive health?

A: I encourage young doctors who want to specialise in reproductive health; many research questions are still far from being fully elucidated, many diagnostic as well as therapeutic approaches could be implemented, and the care of our patients should be improved. Young doctors are the future of the research and of clinical innovation.

Q: How do approaches to fertility and reproductive health differ across Europe? Do you think other countries could learn anything from the Italian approach?

A: I think that currently the most important element to address is the standardisation of women's healthcare and training for all young specialists across European countries, as recently stated by Dr Tahir Mahmood (President of European Board and College of Obstetrics and Gynaecology [EBCOG]) during the 24th EBCOG Congress.

Q: How can patients take better care of their own reproductive health? Can the government or other bodies do more to increase awareness of warning signs to look out for, or ways to prevent common disorders?

A: Specialists in reproductive health should always involve the patients in clinical decisions. European governments should support these processes and increase funds to improve women's healthcare.

Q: What is your opinion on the recent trend in fertility tracking apps?

A: I think that they might be helpful, although they cannot substitute the clinical approach.

Q: What cultural and lifestyle factors have the biggest impact on fertility? What can be done to address these issues?

A: Accumulating evidence suggests that environmental as well as nutritional factors can affect fertility. In this regard, it is an important duty of specialists in reproductive health to continue to provide clear information about these topics to guide the correct patients' lifestyle.

Q: Can you tell us a bit more about your current research and your professional goals for 2016?

A: The next year may be a great turning point for my activities, but many variables have to align in the right way in order to allow me to achieve the desired results.

Q: Are there any areas of research that you hope to explore in the future, which you have not yet had a chance to study?

A: It would be a pleasure for me to start to study the interactions between the different subtypes of macrophages and the endometriotic cells in peritoneal fluid and endometriotic tissue.

> "...it is an important duty of specialists in reproductive health to continue to provide clear information about these topics to guide the correct patients' lifestyle."

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FERTILITY PRESERVATION FOR TRANSGENDER PEOPLE PRIOR TO TRANSITION SURGERY

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Hormonal and surgical treatments for transgender people have a devastating effect on the possibility for these patients to reproduce. Additionally, transgender people tend to start sex reassignment treatment at a young age, when reproductive wishes are not yet clearly defined nor fulfilled. The most recent Standards of Care of the World Professional Association for Transgender Health recommend clearly informing patients regarding their future reproductive options prior to initiation of treatment.[1] Transgender patients are therefore a growing patient population visiting our fertility centres for advice on how to fulfill their future wishes for children or seeking fertility preservation treatments. Where genital reconstructive surgery definitely results in sterility, hormone therapy on the other hand also has an important, but partially reversible impact on fertility. The current fertility preservation options for transgender men are embryo cryopreservation, oocyte cryopreservation, and ovarian tissue cryopreservation. For transgender women, sperm cryopreservation, surgical sperm extraction, and testicular tissue cryopreservation are possible. Although certain fertility preservation techniques could be applicable in a standardised manner based on clear biological criteria, the technique that eventually will be performed should be the preferred choice of the patient after extended explanation of all possible options.

There are a number of options that are available for transgender people, both now or possibly in the future which are discussed in a recent review of our group by De Roo et al.[2] Although theoretically, both transgender women and men are capable of using fertility preservation treatments, there is still an important psychological burden for transgender women to masturbate in order to produce sperm, or for transgender men to undergo repeated endovaginal ultrasound monitoring to eventually collect oocytes for subsequent vitrification. At Ghent University Hospital, the use of *in vitro* maturation (IVM) of oocytes collected at the time of transition surgery upon cryopreservation of ovarian tissue is being explored as a future promising realistic fertility preservation technique for transgender men. Preservation and freezing of ovarian tissue is often chosen as fertility preservation technique by young transgender men: no additional controlled ovarian stimulation by vaginal ultrasound is needed and oophorectomy is standard, at least in Belgium, during sex reassignment surgery. Our research has shown that during the manipulation of the ovarian tissue, cumulus-oocyte complexes can be collected and *in vitro* matured up to the metaphase II stage as is the case in oncofertility preservation programmes. Immature oocytes can also be obtained via endovaginal ultrasound guided aspiration of small follicles on the *in situ* ovaries and matured after collection. The latter is known as IVM. The final goal of our research would be to offer IVM of oocytes obtained from processing ovarian tissue during ovarian cryopreservation (IViMOVA) to transgender men; however, an in-depth examination of the biological function of the IViMOVA oocytes is of the utmost importance as it is unclear what the health of the oocytes are, because they are harvested at a timing where the ovary is residing in supraphysiological levels of testosterone. At the moment it is clear that the IViMOVA oocytes after 48 hours of IVM display a normal spindle structure and chromosome alignment. They can be vitrified and after warming show normal survival rates and normal spindle structures. At least from a morphological point of view, the

supraphysiological doses of testosterone do not have an effect on the IViMOVA oocytes. We are in the process of analysing fertilisation capacity and embryo developmental capacity of the IViMOVA oocytes. This technique, although highly experimental at the moment in transgender men, could definitely maximise fertility preservation options for transgender men and give them a real option for fulfilling their future child wishes with their own gametes.

There is a clear interest in the fertility preservation of transgender people in the assisted reproductive technology (ART) community. Although not possible in all European Union countries because of national legislation, it is of utmost important that patients receive clear information on what their options are. During this presentation at ESHRE, there was a distinct interest in the audience for the general concept of fertility preservation in transgenders, however it is clear that some centres are compromised in storing or using the stored gametes or embryos for treatment, because of national legislation. A healthcare professional stated that from a patient-centred care perspective, the patients could maybe at least store their gametes and embryos and could in the future transport them to countries that do allow the use of these gametes.

Despite the pervasive discrimination and invisibility, in recent years transgender people have experienced significant advances in social acceptance; media attention of the transition of certain celebrities has undoubtedly had an effect. Reproductive transgender care is a true niche in the ART community, however it is obvious that more and more transgender people are 'coming out' and these patients will inform themselves at ART centres regarding reproductive wishes.

I kindly invite you all to share views and practices on reproductive transgender care at ESHRE 2017 in Geneva, where we have a pre-congress course to which top specialists from expert centres in transgender care such as endocrinologists, psychologists, gynaecologists, surgeons, and social researchers are invited.

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THE EFFECT OF GV1001 AND BEVACIZUMAB COMBINATION THERAPY ON FOLLICLE LOSS OF OVARY

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Premature ovarian failure and infertility are serious side effects of cytotoxic drugs in young female cancer patients. A patient's age and early exposure to chemotherapy both influence ovarian reserve, eventually causing primordial follicle loss. Current options for protecting ovarian function include cryopreservation of oocyte, embryo, or ovarian tissue, which require time for ovulation induction. However, delaying treatment is often not possible. Several agents are under investigation that can possibly minimise cytotoxic-induced ovarian damage. New agents such as AS101, imatinib, or anti-Müllerian hormone have been studied to possibly decrease chemotherapy-induced ovarian damage when used in conjunction with cytotoxic drugs. In lieu of searching for new medication, our study investigated the combination effect of GV1001 and bevacizumab on ovarian follicle numbers in mice.

GV1001 was first developed as an anti-cancer vaccine derived from human telomerase reverse

transcriptase. When injected, GV1001 is presented as a major histocompatibility complex (MHC) Class II peptide and endogenously processed to an MHC Class I peptide, stimulating both CD4+ and CD8+ cells to induce tumour cell lysis. There are several Phase I/II trials conducted in patients with pancreatic cancer, advanced melanoma, or non-small cell lung cancer. Most of the studies have focussed on its anti-cancer effects, but in 2014, one study found that GV1001 has a protective effect against renal ischaemia injury by reducing inflammation and apoptosis. If GV1001 can reverse the ischaemia injury, can it also have any potential effect against chemotherapy-induced ovary dysfunction?

Our study was an experimental animal study with 12 mice for the preliminary study and 60 mice for the later study. The preliminary study included four groups; control, GV1001 receiving 2 mg/kg, bevacizumab 5 mg/kg, and GV1001 and bevacizumab combination groups. The medications were injected for a total of six injections over 3 weeks and at the completion of the study, tumour size, follicle counts, primordial follicle rate, and growing ratios were compared. For tumour volume, the GV1001 group showed a greater increase in tumour size compared with the control, which was statistically insignificant. The bevacizumab group and the combination group showed much less change in the tumour size than the control. Regarding ovarian follicle counts, GV1001 group showed a slight decrease compared with the control. With bevacizumab, the follicle count dropped to almost half of the control count. However, when GV1001 and bevacizumab were applied together, the follicle number was found to be similar to the control. For bevacizumab, there were more primary and secondary follicles than there were for the primordial follicles. It was the opposite for the GV1001 and bevacizumab combination group, which showed a higher number of primordial follicles than the primary and secondary follicles. In regards to the growing ratio, bevacizumab showed the highest growing ratio whereas the combination group showed a growing ratio similar to the control group. In order to validate our preliminary results, we increased the total number of mice to 60 and included two more groups using 0.5 mg/kg of GV1001 instead of 2 mg/kg. The primordial follicle counts were similar to the first experiment where GV1001 group showed a decrease in primordial follicle numbers. Interestingly, 0.5 mg of GV1001 showed a smaller decrease compared to the 2 mg group. The bevacizumab group also showed significant decrease in the primordial follicle number compared with the control. Regarding the growing ratio, bevacizumab group was highest and 0.5 mg GV1001 group showed lowest growing ratio.

Our study showed that bevacizumab can cause a significant reduction in the primordial follicle number but not the primary and secondary follicles. These findings suggest that bevacizumab may accelerate primordial follicle activation to cause burn out effect, leading to primordial follicle depletion. Our data also showed that the negative effect of bevacizumab can be reversed with GV1001. The combination of GV1001 and bevacizumab showed a follicle number and growing ratio similar to the control. There seems to be a dose effect where 0.5 mg/kg of GV1001 seems to have a higher preservation effect than 2 mg of GV1001.

There are many questions still to be answered. Why did we see an increase in tumour volume when GV1001 was applied? This may be due to the fact that we used nude mice, meaning that there were no T cells available for tumour lysis. If GV1001 has a protective effect on ovarian reserve, but has increasing effect on tumour volume, then GV1001 is not clinically applicable. This is something that must be clarified in future studies. The most important questions remain; how does bevacizumab activate ovarian follicle development? And how does GV1001 inhibit this activation? We do not yet know.

In conclusion, our study shows that bevacizumab can reduce primordial follicle number through follicle activation and GV1001 can possibly diminish the negative effect of bevacizumab on ovarian follicle loss. More research must be done in order to provide definite evidence regarding the effect of bevacizumab and GV1001 on ovarian function.

ACTIVIN A PRODUCTION AND ACTIONS DURING THREE-DIMENSIONAL CULTURE OF MACAQUE OVARIAN FOLLICLES: A POTENTIAL NON-INVASIVE BIOMARKER FOR FOLLICLE AND OOCYTE GROWTH *IN VITRO*

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Activin A is produced by developing follicles in the adult ovary and has intra-ovarian roles in regulating cell proliferation and steroidogenesis. Activin A actions during early follicular development were studied previously using ovarian tissue or follicle cultures. However, activin A effects during the antral stage have been primarily studied using a granulosa cell culture. We developed a three-dimensional culture technique to grow individual primate preantral follicles to the small antral stage, achieving follicular function in steroid and paracrine factor production. Therefore, studies were designed to investigate the protein production and direct actions of activin A in primate follicles developed *in vitro*.

Rhesus macaque (*Macaca mulatta*; N=4; 4–7 years old) ovaries were collected at the early follicular phase (Days 2–4 of the menstrual cycle). Mechanically isolated secondary follicles (diameter = 125–225 µm; 36–48 follicles/monkey) were encapsulated into alginate (0.25% w/v) and cultured at 5% O_2 for 5 weeks in alpha minimum essential medium supplemented with recombinant human follicle-stimulating hormone and insulin. Follicles were randomly assigned to two groups: A) media-only control and B) 50 ng/mL recombinant human activin A during Weeks 3–5 (after antrum formation). Parameters examined included follicle survival and growth, media activin A (control group only) and steroid (oestradiol and progesterone) concentrations, and oocyte sizes.

In the control group, macaque follicles that survived to grow reached the small antral stage (diameter = 0.5–1.5 µm) at Week 5. The *in vitro*-developed small antral follicles were divided into two distinct cohorts based on their production of activin A. While activin A remained undetectable (<40 pg/mL) in the media for 72% follicles throughout 5 weeks of culture, 28% follicles started to produce activin A at Week 1 with media concentrations increased at Week 5 (40 versus 264 pg/mL; $p<0.05$). Diameters of follicles and their enclosed oocytes were greater at Week 5 in follicles producing activin A relative to those without activin A production (847 versus 736 µm; 112 versus 108 µm; $p<0.05$). While exogenous activin A addition during Weeks 3–5 did not alter follicle survival, activin A exposure decreased diameters of *in vitro*-developed small antral follicles and their enclosed oocytes at Week 5 compared with controls (672 versus 777 µm; 87 versus 109 µm; $p<0.05$). Follicles cultured with activin A during Weeks 3–5 had lower media concentrations of oestradiol and progesterone compared with controls at Week 5 (2,377 versus 3,324 pg/mL; 9 versus 80 ng/mL, respectively; $p<0.05$).

Thus, activin A is produced heterogeneously by primate small antral follicles developed *in vitro* and appears to be a local regulator of follicular development and function in primates. While follicular activin A production correlated positively with follicle and oocyte growth rates, supraphysiologic levels of exogenous activin A suppressed antral follicle development and function *in vitro*. Active activin A production by

small antral follicles may serve as a non-invasive biomarker indicating the potential of follicle and oocyte growth *in vitro*. The findings may be applied to human *in vitro* follicle maturation by selecting follicles for further culture or oocyte maturation to facilitate assisted reproductive technology.

Acknowledgments

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EVIDENCE OF *NLRP7* MUTATIONS IN AETIOLOGY OF MULTILOCUS METHYLATION DEFECTS OF IMPRINTED GENES IN SPONTANEOUS ABORTIONS FROM WOMEN WITH RECURRENT PREGNANCY LOSS

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INTRODUCTION

Recurrent pregnancy loss (RPL) is a hallmark of human reproduction affecting about 1–2% of couples suffering from the occurrence of three or more consecutive losses of pregnancies prior to Week 20 of gestation. The causes of RPL are complicated and often remain unexplained for the couple. One of the most frequent causes for pregnancy loss is chromosomal abnormalities in spontaneous abortions.[1] However, the aetiology of repeated miscarriages with normal karyotype is still elusive from the genetic point of view. One can consider that epigenetic mechanisms should be taken into account. Genomic imprinting is an epigenetic phenomenon, which is related to differential parent-of-origin expression of a subset of genes in the human genome. Recently, a new category of genes was identified that can be involved in regulation of imprinted gene expression.[2,3] The aim of our study was to search for mutations in the *NLRP7* gene (MIM 609661) in spontaneous abortions with normal karyotype and multilocus methylation defects (MLMD) of imprinted genes from women with RPL.

MATERIALS AND METHODS

All 13 exons of *NLRP7* gene were sequenced in 29 spontaneous abortions with MLMD from women with RPL and 7 spontaneous abortions from women with sporadic miscarriage. Epimutations of *PEG1, PEG3, PEG10, DLK1, PLAGL1, KCNQ1OT1,* and *GRB10* genes were detected by microarray analysis and confirmed by methylation-specific or methylation-sensitive polymerase chain reaction in replicative study.

RESULTS AND DISCUSSION

We have reported eight novel missense or frame-shift mutations in the *NLRP7* in seven spontaneous abortions from women with RPL.[4] Mutations were presented in compound heterozygous state with some known polymorphic variants or in homozygous state. The parent-of-origin analysis has unexpectedly revealed that novel mutations in three embryos were inherited from fathers, whereas all polymorphic variants had maternal origin. Significantly, the majority of MLMD were presented by postzygotic hypomethylation of maternal alleles of imprinted genes *PEG3, PEG10, DLK1, PLAGL1,* and *KCNQ1OT1,* whereas germline hypermethylation of paternal alleles of the paternal expressed gene, *PEG1,* was found

exclusively in spontaneous abortions harbouring the *NLRP7* mutations of paternal origin.

CONCLUSION

The prevalence of *NLRP7* mutations in the paternal germline provides new insights into molecular mechanisms responsible for the control of genomic imprinting in spermatogenesis and during early human embryo development. Moreover, this finding highlights both the genetic and epigenetic background of the paternal genome within the complex aetiology of RPL.

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IMPACT OF ENVIRONMENTAL ENDOCRINE DISRUPTOR EXPOSURE IN CONTROLLED OVARIAN STIMULATION OUTCOME IN EGG DONORS

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Since the second half of the 20th century there has been an increase in the frequency of reproductive diseases and a decrease in reproductive function throughout the Western world. It is suspected that there should be other contributing factors apart from genetics. Exposures to compounds related to industrial development might be responsible for these trends. Endocrine disruptors (ED) imitate the effect of biological natural hormones and they could be a cause of reproductive disorders. Several ED have been associated with infertility in women.

Our study question was: Does ED exposure affect the outcome of controlled ovarian stimulation in oocyte donors as a control group of proven fertility? For the evaluation of the stimulation and ovarian response we considered number of days of stimulation, E2 level, measured the human chorionic gonadotrophin daily, daily dose of follicle stimulating hormone (FSH), total dose of FSH, number of antral follicles, number of oocytes retrieval, and number of metaphase II oocytes.

Using ultra performance liquid chromatography coupled with mass spectrometry the following compounds were measured in urine and follicular fluid: caffeine and cotinine (nicotine metabolite); phytoestrogens from soy[a]: genistein and daidzein; plasticisers: bisphenol A and mono-(2-ethylhexyl)-phthalate; and antimicrobials: methylparaben and propylparaben.

Lifestyle exposures to EDs were evaluated using a 40-item questionnaire. A total of 31 female donors were recruited for this study. In summary, the most remarkable findings were:

- Increased caffeine levels in urine and follicular fluid were clearly associated with higher ovarian response
- Chemical exposure had a detrimental effect with a decrease in antral follicles and ovarian response

- Soy[a] consumption resulted in an increased number of antral follicles
- Exercise (walking and cycling) improved ovarian response

We are highly exposed to several ED found in our daily routine work, environment, and food. These compounds could be found in the urine and follicular fluid altering reserve oocyte and ovarian response. Therefore, we suggest that by improving lifestyle and avoiding certain exposures, a better reproductive health and *in vitro* fertilisation outcome could be achieved.

CENTRAL PAIN MECHANISMS RELEVANT TO ENDOMETRIOSIS-ASSOCIATED PAIN

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Traditionally, endometriosis has been thought to generate pain by peripheral processes, either by compressing or infiltrating nerves, or by the associated inflammatory pelvic environment. However, over recent decades we have begun to understand how the central nervous system can modulate and even generate the experience of pain itself, such that the conscious experience of pain is not the same as the peripheral noxious input.[1] Dysfunction in endogenous pain modulatory mechanisms increasingly appears to be important. This presentation reviewed some of the key findings in other chronic pain conditions and considers how they might apply to women with endometriosis-associated pain.

Chronic pain conditions are associated with alterations in both central nervous system structure and function, which are remarkably consistent across conditions. However, it is not completely understood whether these reflect a consequence or a cause of chronic pain, or a combination of both.[1-3] In brief, these include alterations in brain structure (both decrease and increase in volume), function (in response to both noxious and non-noxious stimuli and at rest), chemistry, and endocrine function. Some of these changes reflect clinical features. For example, we previously compared women with dysmenorrhoea, but no other chronic pain conditions to women with non-painful periods. Women with dysmenorrhoea were more sensitive to noxious thermal stimuli on Days 10–12 and 20–22 of their menstrual cycles and showed greater pain-related activation of a brain region called the entorhinal cortex. The amount of activation of this region in response to a moderately painful stimulus related to how intense they rated their usual pain with menstruation.[4] They may also be useful to identify clinical subgroups (phenotypes) that reflect underlying differences in pain mechanisms. For example, a recent brain imaging study suggests that the clinical subdivisons of vulval pain syndrome into primary versus secondary or provoked versus unprovoked are reflected by differences in pain-related brain activity.[5] Moreover, it may be possible to begin to understand the underlying cause of the chronic pain by examining the central changes specific to a condition. For example, we know from experimental models of hyperalgesia that the brainstem plays a key role in amplifying pain[6,7] and that the brainstem also appears to amplify pain in patients with irritable bowel syndrome.[8] Whilst in fibromyalgia there appears to be abnormal dopaminergic neurotransmission in a number of brain regions[9] and a reduction in inhibitory neurotransmission in the insula specifically,[10] a key brain region involved in the experience of pain.

Although a number of studies suggest that women with endometriosis are more sensitive to noxious stimuli than pain-free women,[11,12] to date, there has been little focus on the brain when considering women with endometriosis-associated pain. One structural imaging study suggests that women with chronic pelvic pain whether or not they

have endometriosis, have reduced brain volume. However, perhaps more interestingly, women with endometriosis without pain have an increase in the volume of their periaqueductal gray, a key region involved in endogenous pain modulation.[13] It is possible, therefore, that these women do not feel pain as they are so good at dampening down these signals. Interestingly, in another study, women with endometriosis-associated pain had greater communication between the insula and the medial prefrontal cortex, a key region in endogenous pain modulation.[14] Finally, I presented unpublished data from my group suggesting that communication between key regions of the descending pain modulatory network is increased with increasing duration of pain in women with endometriosis, supporting the need to treat pelvic pain promptly and adequately when it presents, even if an underlying diagnosis has yet to be established.

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COMPREHENSIVE CHARACTERISATION OF THE HUMAN BLASTOCYST miRNome FROM THE INNER CELL MASS, THE TROPHECTODERM, AND THEIR RELATED *IN VITRO* FERTILISATION SPENT CULTURE MEDIA

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INTRODUCTION

The predictive power of blastocyst implantation that is guaranteed by 24-chromosome aneuploidy testing on trophectoderm (TE) biopsies accounts for 50–60%.[1,2] However, several other biomarkers of embryo reproductive competence still need to be investigated. MicroRNAs (miRNAs) are small non-coding RNA molecules acting as ubiquitous and pleiotropic regulators of gene expression.

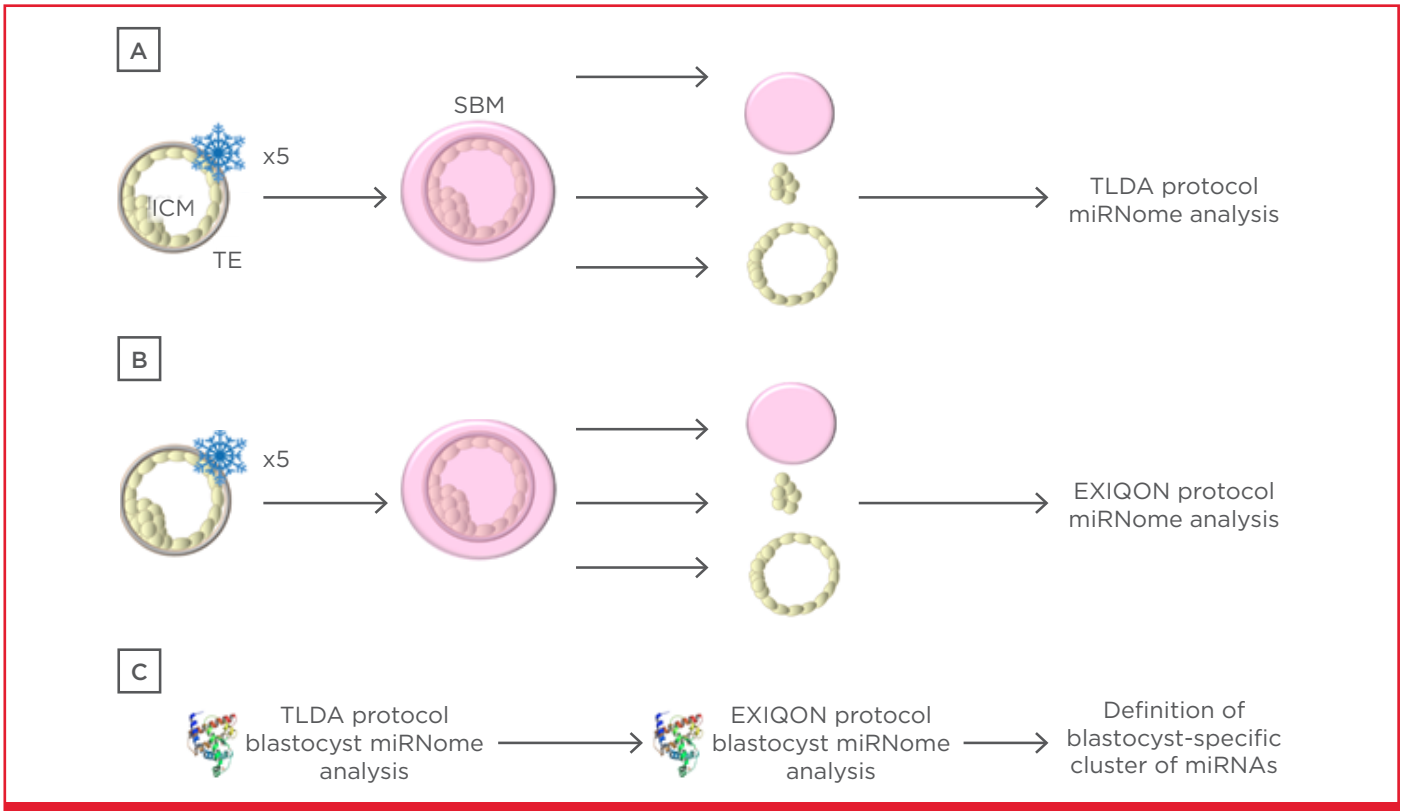

Figure 1: Study design.
A) In the first phase of the study, five blastocysts donated for research were warmed and cultured for 12 hours. Then, the SBM was collected and the ICM was biopsied. The samples composing the five TRIOs were independently analysed according to the TLDA protocol.
B) In the second phase of the study five different TRIOs were created and analysed according to the EXIQON protocol.
C) The data obtained following the two different protocols were compared in order to define a cluster of blastocyst-specific miRNAs validated through two different protocols in two different sets of samples.
ICM: inner cell mass; TE: trophectoderm; SBM: spent blastocyst media; TLDA: TaqMan® Low Density Array; miRNA: microRNA; TRIOs: ICM+TE+SBM.

They can be secreted in the extracellular environment and act into recipient cells.[3] Indeed, they seem to be involved in the blastocyst-endometrium dialogue aimed at implantation[4] and possibly mediate the autocrine communication between the cells within the blastocyst. We recently profiled secreted-miRNAs in spent blastocysts media (SBMs) during *in vitro* fertilisation (IVF). Two miRNAs (*miR-20a-5p* and *miR-30c-5p*) are significantly more abundant in SBMs from implanted versus not implanted blastocysts.[5] miRNAs are thus putative biomarkers of implantation and it is crucial to define their origin and role in preimplantation development.

MATERIALS AND METHODS

This was an experimental study conducted at the Assisted Conception Unit, King's College London, London, UK, between April 2014 and December 2015, and licensed by the NHS Research Ethics Committee. After warming, ten excellent-quality blastocysts donated from consenting couples were singularly cultured in 25 µL microdrops of Quinn's Advantage™ blastocyst medium (ORIGIO, USA) in a humidified atmosphere (5% O_2, 6% CO_2) for 12 hours. After SBM collection, the inner cell mass (ICM) was biopsied using a previously validated method.[6] Five TRIOs (ICM+TE+SBM) were processed with the Taqman® Low

Density Array (TLDA) cards protocol (magnetic beads-based isolation, targeted retrotranscription and preamplification, and Taqman probes-based quantitative polymerase chain reaction [PCR]; human miRNA panel A+B, Thermo Fisher Scientific, USA) by setting the validity criteria as cycle threshold (Ct) value ≤35 cycles in at least three out of five samples from each biological group. Data were confirmed in the other five TRIOs through the EXIQON LNA™ protocol (column chromatography-based isolation, universal retrotranscription, locked nucleic acids® primers and SYBR® GREEN-based quantative PCR; miRNA PCR human panel I+II, EXIQON, Denmark) by setting the validity criteria as Ct value ≤39 cycles in at least three out of five samples from each biological group (Figure 1). Eighty-five percent of TLDA and EXIQON panels (n=754 assays) coincide. IVF blank culture media was used as control. Data analysis was conducted through the RealTime StatMiner® Software (Integromics, Spain) and pathway analysis through the DIANA miRPath software.[7]

RESULTS

The raw data were assessed by Pearson's correlation according to the TLDA protocol and ranged from 0.82–0.87 within biological groups (ICMs, TEs, or SBMs-only) and 0.74–0.83 between them. There were 133 miRNAs (range: 127–146) that were found valid in the TEs (overall mean Ct: 29.0±4.1, range: 3.3–34.9), 64 (72–83) in the ICMs (29.3±3.6, 13.0–34.9), and 71 (65–91) in the SBMs (30.0±3.9, 13.9–34.9), according to the TLDA protocol. Forty-nine miRNAs were exclusively expressed by the TEs, 13 and 20 were shared only with the ICMs and the SBMs, respectively. No miRNA was found solely in ICMs or SBMs. Fifty-one miRNAs were shared by all three biological groups. Fifty-five percent (n=28; 95% confidence interval: 41.35–68.65%) of these last miRNAs were confirmed and validated through the EXIQON protocol. Ninety-one pathways were significantly predicted to be regulated by these 28 blastocyst-specific miRNAs: 38.5% involved in biosynthesis, apoptosis, and differentiation, 28.6% in cell signalling and communication, 19.8% in cell growth and cancer, and 13.2% in inflammation and angiogenesis. In particular, the 11 miRNAs significantly differentially expressed between TEs and ICMs were all found to be involved in promotion/inhibition of cell growth and/or stress-response by directly/indirectly influencing cell cycle regulation and apoptosis.

CONCLUSION

In this study we defined for the first time a cluster of blastocyst-specific miRNAs and their putative role within the human embryo. These data represent an initial step towards the definition of miRNAs' role in the mechanisms of differentiation/loss of pluripotency at this stage of preimplantation development.

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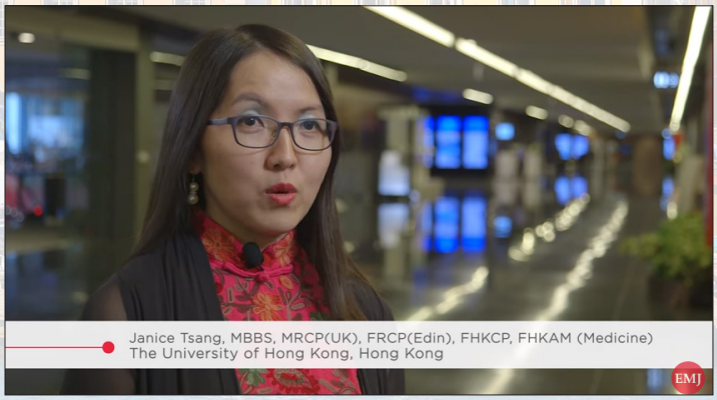

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EDITOR'S PICK

Recruitment to randomised controlled trials (RCTs) is often difficult and this is particularly the case with adolescents and young adults (AYA), because they require a special approach. This fascinating paper by Trent et al. describes the research methods and preliminary effectiveness of recruitment in their trial, and demonstrates that AYA can effectively be recruited and retained to participate in sexual and reproductive health RCTs.

Prof Joep Geraedts

RECRUITMENT OF MINORITY ADOLESCENTS AND YOUNG ADULTS INTO RANDOMISED CLINICAL TRIALS: TESTING THE DESIGN OF THE TECHNOLOGY ENHANCED COMMUNITY HEALTH NURSING (TECH-N) PELVIC INFLAMMATORY DISEASE TRIAL

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ABSTRACT

Purpose: Pelvic inflammatory disease (PID) disproportionately affects adolescent and young adult (AYA) females and can negatively impact their short and long-term reproductive health. Few randomised controlled trials (RCTs) have focussed on strategies to improve outpatient adherence, or to reduce reproductive morbidity in this population. This paper describes the research methods and preliminary effectiveness of recruitment, retention, and intervention strategies employed in a novel RCT designed to test a technology-enhanced community health nursing (TECH-N) intervention among urban AYA females with PID.

Methods: AYAs aged 13–25 years were recruited during acute PID visits in outpatient clinics and emergency departments to participate in this trial, approved by an International Review Board. Participants completed an audio-computerised self-interview, provided vaginal specimens, and were randomised to either standard treatment or intervention. Intervention participants received SMS messaging support for 30 days and a community health nurse interventionist performed a home visit with clinical assessment within 5 days of enrolment. All patients received a full course of medications and completed research visits at 14 days (adherence), 30 days, and 90 days with an outreach worker. Sexually transmitted infection testing was performed at the 30 and 90-day visits. Exploratory analyses using descriptive statistics were conducted to examine recruitment, retention, and follow-up data to test the overall design of the intervention.

Results: In the first 48 months, 63.3% of 463 patients were eligible for the study (293), 81.2% of the eligible patients were recruited for the study (N=238). Most participants were African American (95.6%) with a mean age of 18.6 (standard deviation: 2.3). Of those individuals assigned to the TECH-N intervention, 94% completed the nursing visits. All completed visits were within the 5-day window and over 90% of patients in both arms have been retained over the 3-month follow-up period. Biological data suggests a shift in the biological milieu with the predominance of *Chlamydia trachomatis*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* infections.

Conclusions: Preliminary data from the TECH-N study demonstrated that urban, low-income, minority AYA females with PID can effectively be recruited and retained to participate in sexual and reproductive health RCTs with sufficient investment in the design and infrastructure of the study. Community-based sexual health interventions appear to be both feasible and acceptable in this population.

Keywords: Pelvic inflammatory disease (PID), randomised controlled trials (RCTs), adolescents, minority.

INTRODUCTION

Pelvic inflammatory disease (PID) is a serious reproductive health disorder and disease rates remain unacceptably high among minority adolescent and young adult (AYA) females.[1,2] Each episode of this upper reproductive tract infection, which is usually caused by a sexually transmitted infection (STI), increases the risk of sequelae such as tubal infertility, ectopic pregnancy, and chronic pelvic pain (CPP).[3-8] Care recommendations for PID have shifted from inpatient to the outpatient settings in response to efficacy data from the Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) study.[9] Unfortunately, regardless of the treatment strategy, recent age-stratified analysis from the PEACH data demonstrated that AYAs have high rates of repeat STIs and CPP. Twenty percent of females <25 years old experienced a repeat or persistent STI at 30 days and/or repeat PID over the 7-year follow-up period, with a 5-fold risk of CPP.[10] Furthermore, our local data has shown poor adherence to the 3-day follow-up visit recommended by the Centers for Disease Control and Prevention (CDC), and increased risk for an STI at 90 days. While brief interventions in the acute care setting have had a positive effect, adherence to self-management is problematic.[11]

Inpatient treatment for PID is expensive without incremental increases in effectiveness compared with outpatient treatment,[12-15] therefore cost-effective outpatient PID care supports are needed to improve reproductive health outcomes for this vulnerable population.[10,16,17] Previous work from our group demonstrated that young females perceive significant health-related quality of life reductions and desire more closely monitored care for PID by clinicians, which was informative for strategically guiding the intervention we advanced herein.[14,18]

Research has demonstrated that community health nurse (CHN) interventions can increase access to resources, enhance health care utilisation, and promote risk-reducing behaviour.[19-21] We postulate that integrating a technology component into CHN care would increase appeal to AYAs, given data showing that use of SMS messaging enhances attention paid to medical visits, medication adherence, and health communication.[22,23] We hypothesised that repackaging the CDC-recommended follow-up visit using a technology-enhanced community health nursing intervention (TECH-N) with integration of an evidence-based STI prevention curriculum and SMS communication would reduce repeat infection by improving adherence to PID treatment and reducing unprotected intercourse, and would be more cost-effective compared with standard care.

We are currently enrolling AYAs diagnosed with PID and randomising them to receive CHN clinical support using a single post-PID face-to-face clinical evaluation and SMS communication support during the 30-day period following diagnosis (intervention group) or standard care (control group). In this paper, we discuss the design and methods of this novel, randomised controlled trial (RCT). We also describe the preliminary 48-month effectiveness of recruitment and retention strategies being employed in this research, designed to reduce recurrent STIs that result in adverse reproductive health outcomes after PID.[10,17,24]

CONCEPTUAL FRAMEWORK

This work builds on our previous studies with AYA females with PID demonstrating inadequate

interface with the healthcare system and poor adherence to treatment.[11] For this work, we employ the integrative model of behavioural prediction[25] to frame our approach. Our previous work has allowed us to demonstrate that the distal (demographics) and intermediate (self-efficacy) factors do not sufficiently allow us to predict behaviours. Despite issues with adherence, AYAs express high treatment value and self-efficacy.[26] Capitalising on the positive attitudes towards treatment, we are attempting to change behaviour by:

i) Affirming positive outcome expectations
ii) Enhancing sexual health skills (e.g. condom use)
iii) Removing environmental constraints (e.g. transportation).[9]

We employ comprehensive, structured CHN care that includes:

i) Standardised prevention case-management components
ii) An effective one-on-one intervention for STI behaviour change[27,28]
iii) PID-related health SMS messages as core components of the TECH-N intervention.

METHODS

Study Overview

The TECH-N team is actively enrolling 350 AYAs with mild-to-moderate PID in a 2-arm, single-blinded RCT. Participants are recruited from a large urban academic hospital system located in an STI-prevalent community in the USA. Patients enrolled in Arm 1 (TECH-N intervention group) receive standard care according to the CDC guidelines: a full course of medication, a welcome SMS message followed by daily medication reminders and tri-weekly messages following the 14-day treatment period up to 30 days, and community-based visits by a TECH-N at 3-5, 14, and 30 days following their PID diagnosis. In Arm 2 (control group), patients will also receive standard care treatment including a full course of medications comparable to the intervention group, but are expected to arrange their own 3 to 5-day follow-up, according to standard practice. All participants participate in follow-up interviews at 14, 30, and 90 days. STI testing occurs at the 30 and 90-day study visits, in accordance with timelines for expected disease clearance and reinfection.[29]

Setting and Participants

The study is being conducted in the Baltimore, Maryland metropolitan area, which currently ranks 10th in the nation for incidence of HIV/AIDs infection[29] and 17th for *Chlamydia trachomatis* among its citizens.[30,31] The Maryland Department of Health and Mental Hygiene has determined that every county is a *C. trachomatis* hotspot and county maps demonstrate that Baltimore is the most densely affected.[31] Patients are recruited at the time of diagnosis from paediatric and adult emergency departments, general paediatric departments, and AYA outpatient clinics.

Inclusion and Exclusion Criteria

At enrolment, trained research staff perform a detailed review of the study as a part of the informed consent process and patients are screened for eligibility. Inclusion criteria include age 13–25 years, diagnosis of mild-to-moderate PID with a disposition to outpatient treatment, residence in the Baltimore metropolitan area, willingness to give informed consent, and to be randomised. Patients who are pregnant, have a concurrent diagnosis of sexual assault, or are unable to communicate with staff due to cognitive, mental, or language difficulties are ineligible.

Enrolled participants complete an audio computerised assisted self-interview (ACASI) to collect baseline information, and are randomised to the TECH-N or control group using a permutated block design.[32] Patients also provide a collection of a vaginal swab for STI testing. After completion of initial study procedures and instruction, patients receive remuneration of $10 and a 14-day course of medication dispensed by the clinician.

Technology-Enhanced Community-Health Nursing Intervention

The TECH-N intervention includes follow-up by a CHN trained in clinical assessment, indications for physician referral, disease intervention protocols for partner notification and treatment, the CDC guidelines, and the TECH-N protocol. The CHN uses the Sister-to-Sister Teen® intervention that has a 20-minute one-on-one module to guide the patient through skill-based risk reduction counselling[27,28] during home visits 3–5 days after enrolment, consistent with other published PID trials.[33-35] Given that only 20% of AYAs adhere to follow-up visits,[11] this window proved to be justifiable. Intervention participants are also enrolled in Reify

Health Responsive SMS system® that delivers a welcome message, a prompt to schedule the CHN appointment at 3–5 days, and daily medication reminders for 14 days. After 14 days, the patient also receives positive health reminders for the rest of the month. While 95% of AYAs in our prior research had a cell phone for personal use, many low-income patients have difficulty with monthly maintenance.[22,23] Participants who are assigned to the intervention and do not have a cell phone are provided one for temporary use.

Control Condition

In the control condition, patients receive standard care per CDC guidelines.[36] The institution has integrated standard PID management protocols into usual clinical care as a part of continuous quality improvement protocols.[36]

Disposition

After completion of informed consent, enrolment procedures, baseline ACASI survey, and specimen collection, the clinician dispenses study-supplied doxycycline 100 mg twice daily for 14 days with or without metronidazole 500 mg twice daily for 14 days and provides discharge instructions for self-care. Clinicians also have access to azithromycin 1 g given orally once a week, to be administered with metronidazole 500 mg taken orally twice daily for 14 days through the study, for the rare patient with doxycycline allergy.[9,37]

MEASURES

Interview Data

All participants are interviewed by an outreach worker at 14 days post-enrolment to assess residual symptomatology and adherence to the recommendations for self-care.[9,37] A pill count is performed if the original medication bottle is available at the time of the interview. Patients with ongoing symptoms are discussed with a TECH-N team clinician and referred for care as needed.

In addition to the baseline visit, ACASI is used to collect data at the 30 and 90-day visits. The measures on the baseline survey contain demographics, reproductive and sexual history, PID adherence self-efficacy and perceived barrier scales,[26,7] social provisions scale,[38] and the short-form survey instrument as a measure of health-related quality of life.[39,40] The 30 and 90-day ACASI surveys capture interim sexual behaviour, condom use, contraceptive use, interim diagnosis of STIs and pregnancy, and health status.

Technology-Enhanced Community Health Nursing/Community Health Nursing Visit Data

The CHN nurse records contact tracing (e.g. number of contacts to arrange and complete visit) and clinical assessment data including a pain rating scale, abdominal exam, medication usage, supportive care, side effects, activity level, and patient teaching outcomes.

Costs

The costs of administering the intervention is based on hours worked, miles travelled, other supplies, and overhead. The primary cost components for patients in both arms include the initial treatment for PID, follow-up therapy for treatment failure, treatment for recurrent STIs and PID, CPP, ectopic pregnancy, time lost from work/school/household management, travel to PID-related care, and therapy or evaluation for infertility. These data will be used to estimate the direct (medical costs) and indirect (employment, impairment) costs associated with PID for the TECH-N and control arms. Health service utilisation will be derived from the longitudinal interviews, care utilisation data, prescription and over-the-counter medication use, laboratory services, and radiological services. Productivity loss will be derived using the Workplace Productivity and Activity Impairment Questionnaire.[41,42]

Biological measurements

Participants are asked to provide a self-collected vaginal swab for *Mycoplasma genitalium* and *Trichomonas vaginalis* at baseline to accompany routine testing at the clinical site. Samples to test for *Neisseria gonorrhoeae*, *C. trachomatis*, *T. vaginalis*, and *M. genitalium* are also obtained at 30 and 90 days and processed at the Johns Hopkins University International STD and Respiratory Research Laboratory, Baltimore, Maryland, USA. Vaginal specimens are processed and tested according to the Aptima® Combo 2 (Hologic, Marlborough, Massachusetts, USA) package inserts for *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis*.[43] The Aptima urine-based assay was tested using the Gen-Probe transcription mediated amplification research assay in the same manner as for the other Aptima assays. This assay targets *M. genitalium* r RNA for detection in genital specimens.[44-48]

All participants with positive STI tests are contacted by a research nurse practitioner, notified of their positive test result, and referred for treatment. Positive cases of *N. gonorrhoeae* and *C. trachomatis* are reported to the local health department. The TECH-N encourages partner notification and treatment while providing support and resources to assist all participants.

Incentives

Participants receive a $10 gift card at enrolment, and $10 for each completed face-to-face research visit (14, 30, and 90 days) plus an additional $10 for each STI sample (N=3). AYAs in the TECH-N intervention group do not receive remuneration for the 3 to 5-day clinical care visit because adherence to the CHN clinical visit is a non-incentivised behaviour under study. Participants receive $5 remuneration for notifying us of changes in cell phone number and/or other contact information.

STATISTICAL ANALYSES

Sample Size

Sample size calculations were driven by the results of our prior research using longitudinal data to examine repeat STIs/PID after an initial diagnosis of PID. At 3 months, the STI positivity rate was 25%[24] and thus we assumed this infection rate for the control group. Preliminary data also suggested that we will be able to recruit approximately 175 participants in each arm for a total of 350 participants. We anticipated about 30% attrition over the study period thus each study arm will have an effective sample size of 122.5 participants, for a total of 245 participants.

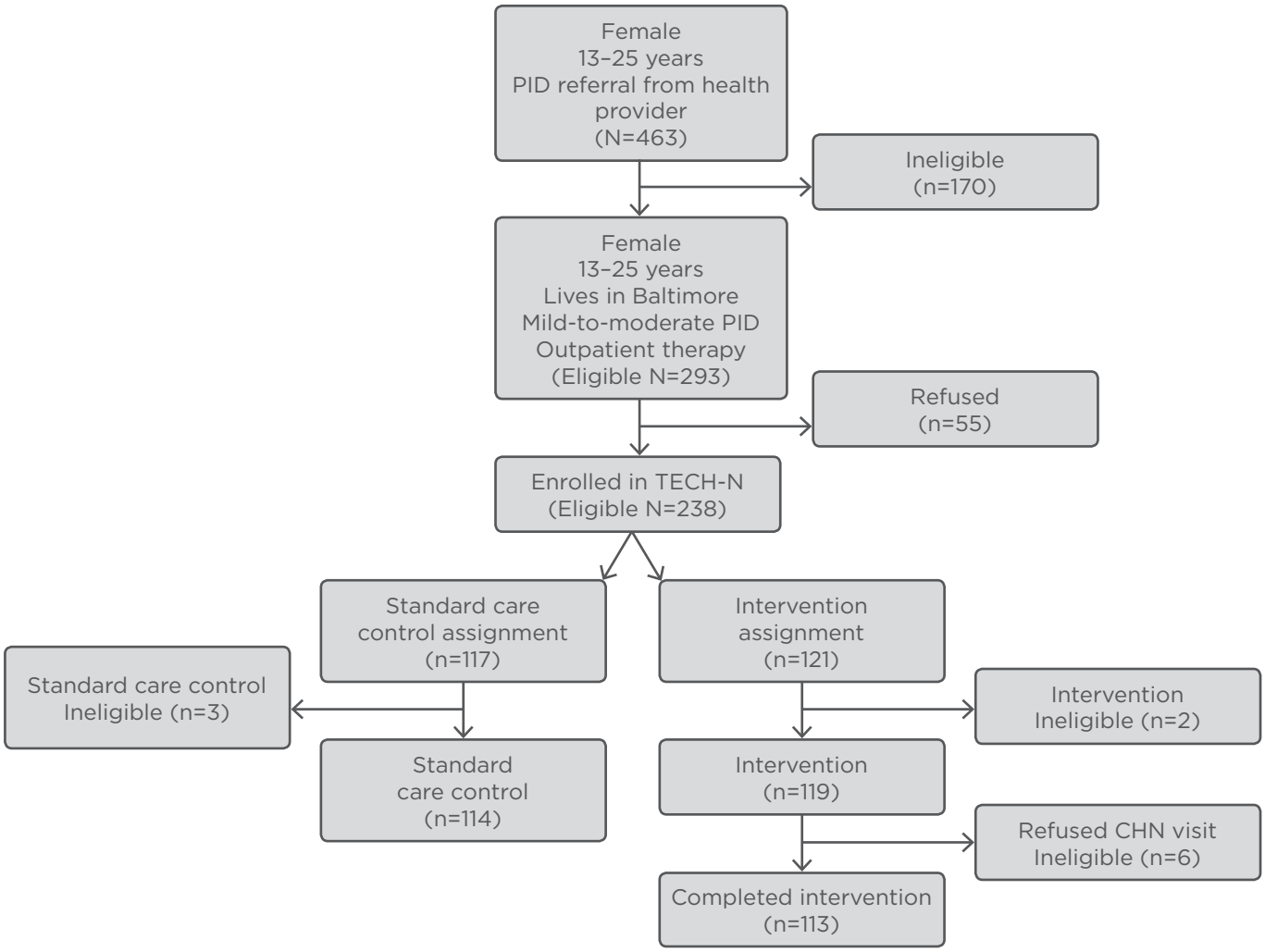

Figure 1: Outcomes of recruitment, randomisation, and retention efforts within the technology-enhanced community-health nursing (TECH-N) study.

PID: pelvic inflammatory disease; TECH-N: technology-enhanced community health nursing; CHN: community health nurse.

Using these assumptions, our power calculations suggest that we will have 80% power to detect a relative risk of 1.66.

Data Analysis

The TECH-N study will compare the effectiveness of the intervention with standard care using an intention-to-treat analysis. We will conduct a logistic regression analysis to: i) determine whether the intervention group showed greater short-term adherence rate compared to the control group and ii) determine whether the intervention group, showed a lower rate of recurrent STIs at 90 days compared to the control group, including potential factors that may affect the difference in rates for both models.

The economic evaluation will examine the net difference in costs between TECH-N and control groups in relation to prevention of: i) recurrent STIs and ii) PID-related complications. The difference in costs will account for the net difference between the outpatient strategies in use of medical services and indirect costs for PID treatment. We will also consider savings in medical-care costs and indirect costs from prevention of the outcome variables. A cost-benefit analysis can be performed comparing the excess costs of TECH-N with the medical care savings and productivity gains from cases averted. We can extend the analysis to a cost-effectiveness evaluation if TECH-N remains more expensive even after accounting for medical care costs and productivity loss averted. In this case, we will measure the dollars spent per case of STI averted over the relevant period.

STUDY UPDATE

In the first 48 months, 293 of 463 patients were eligible for the study (63.3%) and 238 (81.2%) of eligible patients were enrolled (Figure 1). The mean age of participants was 18.6 (standard deviation: 2.3). Most participants were low-income (Medicaid/self-pay [77%]), African American (95.6%), and resided in female-headed households with maternal education level as high school or less. Patients who declined study participation were older (19.8 versus 18.6 years, $p<0.05$), but otherwise were demographically similar. To date, only 14 of intervention patients (11.6%) required a study cell phone for participation. To date, 19 patients were lost to follow-up, but were demographically indistinct (age, race/ethnicity, insurance status) from those who completed the intervention. Of those individuals assigned to the TECH-N, 94% completed the intervention. The average number of contacts to execute the CHN visits was 2.46. Almost all eligible participants completed their 14, 30, and 90-day visits (95%, 96%, and 93%, respectively). Mean number of days to complete the 14-day research visit was 1.1 days (SD: 0.55). Baseline laboratory results reveal infection rates that are highest for *C. trachomatis* (26%) and *M. genitalium* (21%), followed by *T. vaginalis* (16%) and *N. gonorrhoeae* (8%). While *M. genitalium* is associated with PID,[33] recent studies have suggested low rates of infection in adolescents[49] and there is currently no commercial laboratory test for *M. genitalium* to diagnose patients in the USA outside of the research environment. Our data will allow for a longitudinal analysis that may drive differential approaches to testing and therapy.

SUMMARY

PID remains a common reproductive health disorder disproportionately affecting AYA females in urban minority communities. We have demonstrated that using the TECH-N design, AYAs can effectively be recruited during acute PID treatment visits to participate in a longitudinal RCT and can be retained over time. Furthermore, sexual health counselling and clinical follow-up interventions for STI management can be reliably administered in the home by CHNs. While PID is a polymicrobial disorder, the baseline rates of STI positivity suggest a shifting biological milieu in this cohort of AYA females. Moreover, we are reaching the target population of AYAs at high risk for recurrent STIs for intervention.

The strengths of this study include utilisation of evidence-based intervention materials (Sister to-Sister Teen) and innovative communication tools (Reify® SMS) for intervention delivery, and embedding the study in an existing clinical infrastructure. This includes having access to electronic health records to track patient visits during recruitment and the follow-up of results and care outcomes, full-time research staff to work with clinical staff in recruitment sites, and service provision (outreach, STI testing, CHN visits) as a part of the research protocol. As such, TECH-N research staff are viewed as an extension of care.

Staffing of the study has also been a critical component for the success of this programme. In our prior research patients with PID, we

demonstrated high recruitment rates but retained only 70% of the sample.[11] Investment in both recruitment and outreach staff has ensured that patients can be effectively tracked in the field without undermining the onsite team. Staff training and team building has resulted in a knowledgeable and high functioning interdisciplinary team. The team is also diverse, but most field staff members are female, further elevating the 'Sister-to-Sister' concept. Our research staff have been described as personable, culturally sensitive, patient, non-judgmental, supportive, committed, and persistent in terms of their execution of the protocol and follow-through with patients in complex social circumstances.

We also recognise the limitations due to the limited generalisability of our sample as a result of the demographics and epidemiology of STIs in Baltimore.[29-31] Despite this, our work has the potential to identify an alternative, cost-conscious strategy for addressing the observed disparities for AYAs with PID. As with the PEACH trial,[34] the confounding effects of race/ethnicity and socioeconomic status are difficult to parse out because the burden of PID is predominately borne by low-income minority women. The PEACH trial group found that younger aged and lower income females were hard to recruit and often excluded.[34] We actively seek out these women and attempt to overcome the latter by optimising our design.

Ultimately, this research demonstrates that inclusion of urban, minority AYA females in large community-based sexual health RCTs is highly feasible. It also suggests that adequate investments in developing the research infrastructure are critical for the execution of RCTs designed to reduce PID-related health disparities among vulnerable populations in communities with high STI prevalence.

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MATERNAL LIFESTYLE FACTORS AND FETAL MACROSOMIA RISK: A REVIEW

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ABSTRACT

Fetal macrosomia is associated with a number of health complications for both mother and infant in the immediate, short, and long-term. Maternal obesity and excessive gestational weight gain (GWG) have long been associated with fetal macrosomia, however the impact of maternal lifestyle factors such as dietary intake and energy balance, in combination with the timing and composition of weight gain, have been less studied. It is also clear that although maternal obesity and excessive GWG increase the risk of fetal macrosomia independently, the risk is magnified with the presence of both risk factors, suggesting that interventions to control GWG may be particularly important for obese women. Association studies examining the relationship between fetal nutrient availability, epigenetic modifications, and infant anthropometrics are also required. This review provides an overview of the current evidence examining the role of maternal lifestyle factors on the prevalence of fetal macrosomia and identifies areas where further research is required in order to inform the design of appropriate intervention strategies.

Keywords: Birth weight, body composition, gestational weight gain (GWG), macrosomia, maternal obesity.

INTRODUCTION

Birth weight is a key determinant of infant health, which appears to be determined by a complex interaction of maternal and fetal factors. These probably include maternal genetic, environmental, and lifestyle factors, in conjunction with fetal genetic and intrauterine environmental factors. Macrosomia is generally defined as a birth weight >4,000 g or 4,500 g, irrespective of gestational age,[1] while large for gestational age (LGA) is defined as a birth weight >90th percentile as per gestational age.[2]

Excessive fetal growth results in increased immediate, short, and long-term risks for both mother and infant. Macrosomia increases the risk of complications during delivery such as birth asphyxia, shoulder dystocia, and increased incidence of delivery via caesarean section, which carries its own adverse risks to both neonate and mother.[3] Furthermore, higher birth weight is also associated with increased risk of obesity[4] and metabolic syndrome[5] into childhood, which have serious long-term health consequences.

Numerous maternal factors such as body mass index (BMI), gestational weight gain (GWG), diet, physical activity, and the development of gestational diabetes have all been shown to impact infant birth weight. However, previous studies have tended to examine these factors individually, and their interaction even less so. The purpose of this review is to critically appraise the current literature and highlight areas where further research is required to inform appropriate maternal intervention strategies, with the aim of improving neonatal health.

MATERNAL WEIGHT

When examined individually, high maternal pre-pregnancy BMI tends to be strongly associated with an increased risk of macrosomia. Numerous studies have reported women classified by their BMI as obese to be at a significantly greater risk of macrosomia compared with women classified as having a healthy weight,[6,7] with risk increasing as BMI increases beyond the healthy range.[8] A recent systematic review and meta-analysis

conducted by Gaudet et al.[1] showed a positive relationship between maternal obesity and fetal overgrowth as defined by birth weight ≥4,000 g, ≥4,500 g, and ≥90th percentile for gestational age.

Similarly, excessive GWG has been shown to increase risk of macrosomia.[9-11] Although it appears that both maternal obesity and excess GWG independently increase the risk of macrosomia, the interaction between the two factors is less clear. Crane et al.[12] conducted a retrospective cohort study evaluating the effects of GWG on maternal and neonatal outcomes in different BMI classes. In keeping with findings from previous studies[6-8] they observed that overweight and obese mothers were significantly more likely to give birth to a macrosomic infant (birth weight ≥4,000 g and adjusted for gestational age) and also more likely to gain excess weight than healthy weight mothers. However, when the impact of GWG on risk of macrosomic infant was examined by BMI class, risk increased with excess GWG for all BMI classes suggesting that when excessive GWG does occur, the risk of macrosomia increases regardless of pre-pregnancy BMI. A major limitation of this study was that it was retrospective, and so pre-pregnancy BMI or GWG data were missing for 47.8% of the study participants. Nohr et al.[13] conducted a similar study reporting that BMI category was a stronger predictor of LGA neonate than GWG, but that very high GWG (defined as >20 kg) increased the absolute risk of LGA neonate across all BMI categories. Limitations of the study were that pre-pregnancy weight, height, and GWG were self-reported and thus the reliability has been disputed.[14] In addition to examining the effect of maternal obesity and GWG on infant birth weight, Carlsen et al.[15] included neonatal body composition as an outcome measure. They observed that infants born to obese mothers were heavier than infants born to healthy weight mothers, and this was exclusively due to increased adiposity. GWG on the other hand, was found to increase fat mass, abdominal fat mass, and fat-free mass. Obese mothers were more likely to exhibit excessive GWG, thereby suggesting these women as a particularly important target group to receive an intervention with an aim of reducing fetal macrosomia.

The effect of GWG on maternal and neonatal outcomes in women classified as having a healthy pre-pregnancy BMI was examined by Deruelle et al.[16] Although most neonatal outcomes were similar between GWG groups, mean birth weight was significantly greater in women with ≥18 kg GWG than women gaining 9–15 kg, while the proportion of macrosomic neonates more than doubled for women with ≥18 kg GWG compared with those gaining 9–15 kg (12.1% versus 5.2%, p<0.03). Prevention of excess GWG in women of healthy pre-pregnancy BMI is therefore also important, just as in overweight or obese mothers. In 2009, the Institute of Medicine (IOM) published a new set of guidelines on GWG to replace those previously published in 1990[11] and now make recommendations based on pre-pregnancy BMI category for total and rate of weight gain.

It has been suggested that birth weight and early childhood growth patterns can lead to a predisposition to childhood obesity, with the potential to persist into adolescence and adulthood.[17] In a diverse sample of women from the USA, inadequate GWG, when compared with adequate weight gain, was associated with significantly increased odds of infants being born small for gestational age (SGA), while excessive gain was significantly associated with decreased odds of SGA and more than doubled the risk of LGA.[18] Excessive GWG also significantly increased the risk of child overweight or obesity (BMI ≥85th percentile) when followed up between the ages of 2 and 20 years. For overweight and obese women, predicted probabilities of LGA newborns and childhood overweight were higher than those for underweight or healthy weight women, regardless of GWG. Increased GWG was significantly associated with increased probability of LGA and an overweight child across all BMI groups. Similarly, a retrospective cohort of 499 mother–child dyads[19] observed that maternal morbid obesity (BMI ≥40 kg/m^2) was significantly associated with infant birth weight and weight for length throughout the first 3 months of life, and that these associations were significantly amplified by excess GWG. At 12 months of age these effects were sustained, with infants of morbidly obese mothers exhibiting an 8.4% higher weight for length percentile compared with infants of mothers with a BMI of 25 kg/m^2. Infants born to mothers with a healthy BMI but with excess GWG normalised their growth by 12 months of age.

These findings suggest that babies born to women in all BMI categories are at risk of increased birth weight and elevated weight during early life as a result of excessive GWG, but that overweight and obese women are of particular concern, as their risk appears to be amplified.[15,19] Future studies,

particularly of a prospective nature, should therefore focus on this group of women in order to develop a wider understanding of lifestyle factors that contribute to excess GWG.

MATERNAL BODY COMPOSITION

Although BMI is widely used to provide estimates of body composition, it is not without its limitations. Prentice and Jebb[20] propose that obesity should be defined as the excess accumulation of body fat, whereas BMI identifies the presence of excess body weight, which also reflects lean body mass. Krentz et al.[21] compared birth weight outcomes for women with the same BMI, but two different heights in a retrospective cohort study. They observed differences in birth weights and birth weight classification by gestational age between groups, which once again provided evidence to suggest the limited utility of BMI as a predictor of neonatal outcomes. In addition, GWG is typically reported as a single measure of mass gained during pregnancy, with the individual effects of fat mass and fat-free mass gains left undefined. It therefore seems prudent to examine the contributions of changes to estimated maternal fat mass and fat-free mass on pregnancy outcomes, in addition to total GWG and maternal obesity defined by BMI.

As might be expected, maternal weight, fat-free mass, and fat mass increased between 28 and 37 weeks gestation in a recent prospective cohort study examining maternal body composition. However, birth weight significantly correlated with maternal fat-free mass and not fat mass.[22] In a similar study, fat-free mass, but not fat mass, was also a significant predictor of birth weight and after adjustment for confounding variables, mothers in the highest fat-free mass quartile were at significantly higher risk of infant macrosomia, compared with mothers in the lowest quartile.[23] However, this study measured body composition only in the first trimester. Butte et al.[24] divided GWG into fat mass, fat-free mass, total body water, and protein gains as assessed at 9, 22, and 36 weeks of gestation. Infant birth weight was found to correlate significantly with fat-free mass ($r=0.39$, $p=0.003$) and total body water ($r=0.37$, $p=0.006$), but not fat mass ($r=0.05$, $p=0.76$). These studies suggest that fat-free mass, and not fat mass mediates an increase in infant birth weight. It is hypothesised that these positive associations between maternal fat-free mass and infant birth weight may be due to maternal plasma volume expansion,[25] which in turn is influenced by maternal hormonal changes.[26]

Forsum et al.[27] addressed the hypothesis that maternal body fat stimulates fetal growth and fat deposition. In a small, observational study they assessed infant subcutaneous adipose tissue volume *in vivo* using magnetic resonance imaging, while maternal body composition was assessed using a two-compartment model based on total body water. It was observed that maternal total body fat before pregnancy and at 32 weeks gestation was significantly and positively correlated with infant birth weight, while in infants, birth weight positively correlated with subcutaneous adipose tissue. Further studies examining the effects of maternal body composition on neonatal body composition and incidence of macrosomia are therefore required in order to fully understand the relationship between the composition of GWG and infant birth size.

TIMING OF GESTATIONAL WEIGHT GAIN

Although the influence of total GWG during pregnancy has been well documented, the timing of overnutrition and subsequent weight gain has not been examined as thoroughly. This could be an important factor in the design of any intervention studies. Davenport et al.[28] evaluated whether the timing of excessive GWG in pregnant women following current healthy living guidelines affected neonatal adiposity at birth in their prospective cohort study. The cohort was retrospectively grouped according to IOM guidelines[11] by weight gain in the first and second halves of pregnancy. Infants born to women who exhibited excessive GWG during the first half of pregnancy exhibited greater birth weight, crown–heel length, and excessive neonatal body fat compared with infants born to women who exhibited appropriate GWG in the first half of pregnancy. These differences remained significant after controlling for BMI, total GWG, maternal age, gestational age, and neonatal sex. Farah et al.[22] conducted a longitudinal prospective observational study which observed that birth weight was significantly correlated with GWG before the third trimester ($r=0.163$, $p=0.027$) but not with total or third trimester GWG. These studies suggest that neonatal adiposity is potentially more strongly influenced by timing of GWG than total GWG, suggesting a direct link between the early

intrauterine environment and subsequent neonatal adiposity. However, the data on timing of GWG and its influence on neonatal weight and adiposity is limited. Studies examining weight change during pregnancy with frequent assessments are therefore required in order to increase our understanding of the mechanism by which maternal obesity and GWG influence infant birth weight and body composition.

GESTATIONAL DIABETES MELLITUS

Gestational diabetes mellitus (GDM) is a common metabolic complication of pregnancy, defined as glucose intolerance with first onset or recognition during pregnancy.[29] GDM is most frequently observed amongst overweight or obese women[30] as these women are more likely to exhibit impaired glucose tolerance and decreased insulin sensitivity before and during pregnancy[31] when compared with women of a healthy weight. Infants born to women with GDM are often characterised by excessive fetal growth and subsequently tend to be at increased risk of macrosomia.[31] However, even in the absence of increased body mass, studies have shown that infants born to mothers with GDM exhibit increases in fat mass, but not fat-free mass when compared with women with normal glucose tolerance.[32,33] Results from the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study observed an increase in neonatal adiposity associated with increasing maternal glucose concentrations, less than those used to define GDM.[34] Physical activity has also been shown to influence glucose metabolism and transport via insulin-independent pathways and has been associated with a decreased incidence of GDM in epidemiological studies.[35]

MATERNAL ENERGY INTAKE AND EXPENDITURE

Clearly, nutritional status prior to and during pregnancy is essential for the growth and development of the fetus, with excessive GWG and adverse pregnancy outcomes also largely influenced by dietary intake either as nutrient excess, nutrient deficiencies, or by indirectly influencing the intrauterine environment. A study by Knudsen et al.[36] supports the theory that maternal glucose metabolism may impact fetal growth. They examined the associations between maternal glycaemic load, GWG, birth weight, and risk of LGA neonate as part of the Danish National Birth Cohort. They observed that the risk of LGA neonate increased by 14% for the highest glycaemic load quintile, compared with the lowest quintile. A randomised controlled trial examining the impact of a low glycaemic index diet on neonatal anthropometry observed a decrease in neonatal thigh circumference for the intervention group when compared with a control group, although no differences were observed for any skinfold measurements, nor head, abdominal, and mid-upper arm circumferences.[37]

In a prospective study, GWG was significantly and positively associated with energy intake and energy-adjusted intakes of lipids from animal origin and protein, while a significant inverse association was observed between carbohydrate intake and GWG, but these were not significantly related to birth size.[38] Olsen et al.[39] observed that milk consumption during pregnancy was inversely associated with SGA, and directly associated with LGA and mean birth weight. Women consuming ≥6 glasses of milk/day had increased risk of LGA infants when compared with women who reported no milk consumption. When fat and protein intakes from dairy products (excluding cheese and ice cream) were examined, no association between birth weight and fat intake was found, while a positive association between protein intake and birth weight was observed. The authors proposed that the positive association between milk consumption and birth weight is driven by the presence of insulin-like growth factor 1 in both low-fat and whole-milk products. Montpetit et al.[40] examined the contribution of pre-pregnancy BMI, energy intake, and physical activity as determinants of GWG and infant birth weight. Energy intake was the only significant predictor of infant birth weight. Steps per day were inversely associated with GWG, although when pre-pregnancy BMI was added to the model, steps were no longer significant and BMI remained the only significant variable.

A study conducted in the USA[41] observed decreases in birth weight and LGA births between 2000 and 2005, trends which did not appear to be explained by routinely recorded maternal characteristics. The authors hypothesised that other maternal characteristics such as maternal diet, physical activity, or socioeconomic factors may have contributed to the trends observed and called for detailed studies of smaller populations to explore the role of these factors.

Furthermore, the rapidly expanding field of epigenetic epidemiology has observed numerous associations between fetal nutrient availability and epigenetic modifications.[42] Differences in the methylation status of candidate genes have been observed in relation to fetal growth[43] and later childhood adiposity.[44,45] However, human studies examining specific intrauterine nutritional exposures and subsequent adiposity at birth and during childhood are scarce. Studies of an observational and epigenetic nature are therefore essential for increasing our understanding of how nutritional exposures influence GWG and infant phenotypic outcomes.

CONCLUSION

It is important to gain an understanding of the factors influencing neonatal anthropometric outcomes, as macrosomic infants with or without excess adiposity at birth have been shown to be at increased risk of adverse consequences such as insulin resistance,[46,47] metabolic syndrome,[5] and childhood obesity.[4,48] As observed in the current literature, there is consistent evidence to suggest that maternal obesity and excess GWG alongside GDM contribute to increased risk of adverse neonatal anthropometric outcomes;[12,13] hence current pregnancy interventions are already aiming to reduce the prevalence of these risk factors. However, maternal obesity and GWG are broad outcome measures. Recent studies suggest maternal body composition and timing of GWG may influence infant anthropometrics independently of maternal BMI and total GWG, which may offer an increased understanding of the mechanisms by which maternal obesity and GWG influence neonatal anthropometric outcomes. At present, data in this area is limited[22,23,28] and there is also a lack of recent prospective studies examining the effects of GWG by BMI according to the most recent IOM recommendations.[11]

Table 1: The contributions of maternal lifestyle factors to risk of macrosomia.

	Increased risk of macrosomia/LGA/higher birth weight		Unaffected risk of macrosomia/LGA/higher birth weight	
Factor	**Evidence?**	**References**	**Evidence?**	**References**
Maternal pre-pregnancy BMI 30 kg/m^2	Yes	1,6-8,18	No	N/A
GDM	Yes	31-34	No	N/A
Excess total GWG	Yes	9,10,16,18	Yes	22
Maternal obesity and excess total GWG	Yes	12,13,15,19	No	N/A
Early excessive GWG (first or second trimester)	Yes	22,28	No	N/A
GWG in third trimester	No	N/A	Yes	22
Maternal fat mass	Yes	27	Yes	22,24
Maternal fat-free mass	Yes	22-24	No	N/A
Dietary energy intake	Yes	40	Yes	38
Dietary fat intake	No	N/A	Yes	39,51
Dietary protein intake	Yes	39	No	N/A
Milk consumption	Yes	39	No	N/A
Glycaemic load	Yes	36,37	No	N/A
Physical activity	No	N/A	Yes	8,40

BMI: body mass index; LGA: large for gestational age; GDM: gestational diabetes mellitus; GWG: gestational weight gain; N/A: not applicable.

Maternal diet and energy balance during pregnancy undoubtedly influence GWG and subsequent anthropometric outcomes for offspring. However, despite a wealth of studies linking maternal energy intake to GWG,[38,49] and maternal dietary glucose intake to neonatal anthropometry,[36,37] few studies have examined the impact of other nutrients in the maternal diet, nor energy balance together with physical activity. Studies examining nutritional exposures during pregnancy and epigenetic modifications in offspring are also required.[50]

The contributions of various maternal lifestyle factors to fetal macrosomia from the current literature are summarised in Table 1. As discussed however, there are gaps in the current literature, as well as conflicting findings. It is therefore necessary to examine further the independent and moderating effects of maternal dietary intake, physical activity, and the timing and composition of GWG on neonatal anthropometric outcomes in future studies. Such studies could provide a more complete picture of the maternal lifestyle factors contributing to GWG, neonatal body composition, and potentially future offspring health, thus allowing health professionals to develop suitable and effective interventions to improve birth and health outcomes for both mother and infant. In the meantime, pregnant women should be advised to adhere to IOM guidelines for weight gain[11] and offered nutritional support if necessary. Particularly close attention should be paid to women entering their pregnancy with a BMI ≥30, as offspring of these women appear to be at increased risk of macrosomia, regardless of the contribution of other potential risk factors yet to be investigated.

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ROBOT-ASSISTED LAPAROSCOPIC RADICAL PROSTATECTOMY: A REVIEW OF TECHNIQUE AND OUTCOMES

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ABSTRACT

There has been an increase in the incidence of prostate cancer over time, and it now constitutes 15% of all male cancers in developed countries and 4% in developing countries. Radical prostatectomy is the gold-standard treatment choice in cases determined with organ-confined prostate cancer and life expectancy is >10 years. Currently in the USA, 80% of radical prostatectomy operations are robot-assisted. Although there is an additional financial burden in comparison with open surgery radical prostatectomy, the early recovery of functional status of continence and potency seem to be major advantages of the robotic method. However, there is clearly a need for future prospective studies of large patient series with longer follow-up to further clarify the financial issues, and the oncological and functional status.

Keywords: Prostate cancer, robot-assisted laparoscopic radical prostatectomy, trifecta.

INTRODUCTION

Prostate cancer is the most commonly seen solid neoplasm, and is the second most common cause of death in males in the USA. In 2015, approximately 220,800 individuals were diagnosed with prostate cancer and of those 27,540 were reported to have died from the disease.[1] Together with an increase in the incidence of prostate cancer over time, it constitutes 15% of all male cancers in developed countries and 4% in developing countries.[2] Since the introduction of the use of prostate specific antigen in clinics in 1987, the incidence of metastatic prostate cancer has been reduced and the incidence of organ-confined prostate cancer has significantly increased.[3]

Radical prostatectomy is the gold-standard treatment choice in cases determined with organ-confined prostate cancer and life expectancy is >10 years. The primary aim of the operation is to completely remove the tumour. However, significant attention must be given to the patient being able to postoperatively maintain an erection and continue life without urine leakage.[4] At the beginning of the 1980s, Walsh and Donker[5] first described radical retropubic prostatectomy. Although the oncological results were positive, the feeling was that there was a need for more minimally invasive methods because of perioperative complication rates or postoperative problems such as erectile dysfunction and incontinence. In the early 1990s, Schuessler et al.[6] described laparoscopic radical prostatectomy, although this did not come into widespread use because of difficulties in the technique and a long learning curve.

The introduction of the da Vinci robotic system, with its capacity for hand and wrist type movements, thereby eliminating surgeon hand tremor, provided the possibility of successful surgical dissection and anastomosis; the system allows the ability to work in a more sensitive three-dimensional environment and thus it has been possible to reduce the difficulties of the complex laparoscopic method.[7,8] The first robot-assisted laparoscopic radical prostatectomy was performed by Binder and Kramer[9] in 2000. In 2001 they published the results of 10 cases of robot-assisted laparoscopic

radical prostatectomy. Since then, the use of the da Vinci robotic system has become rapidly widespread. Currently in the USA, 80% of radical prostatectomy operations are robot-assisted.[10]

COMPLICATIONS

Early and late-stage complications may be seen following a radical prostatectomy. Early complications include bleeding, ureteral injury, intestinal injury, deep vein thrombosis, myocardial infarction, pulmonary embolism, and death. Late complications are incontinence, erectile dysfunction, and anastomotic stenosis.[11,12] Many studies have evaluated the perioperative and postoperative complication rates of radical prostatectomy after the development of minimally invasive surgery and have compared these with open procedures. The mean total complication rate in the minimally invasive robotic procedure has been shown to be lower.[13-15] In a study of 20,000 cases, which compared the robotic method with the open method, the former was shown to be advantageous in all the perioperative data.[16]

Some authors have reported significant advantages of minimally invasive surgery compared with open procedures, especially with respect to perioperative bleeding and postoperative transfusion rates.[17-19] It has been emphasised that this advantage can be attributed to a better and more detailed view provided by the magnified image of the laparoscopic camera in the minimally invasive method and the suppression of bleeding, especially venous leakage, due to the tamponade effects produced by the formation of a pneumoperitoneum.[17,18] Similarly in a meta-analysis by Novara et al.,[20] all the perioperative complication rates were reported to be lower in robotic surgery than in open surgery, although statistically significant differences were only observed in the amount of blood loss and blood transfusion rates. Another important perioperative statistic is the operating time.

When it is considered that the patient is in the maximum Trendelenburg position in the robotic procedure, the operating time is of greater importance. Sugihara et al.[15] compared robotic radical prostatectomy with both open surgery and laparoscopic surgery, which showed that the duration of anaesthesia was 42.6% and 6.9% longer than in the other two methods, respectively. However, this prolonged period of anaesthesia was not reported to increase the complication rates. Recent studies have shown that with the widespread use of the robotic system and completion of the learning curve by surgeons, operating times have significantly reduced and have been shown to be similar to those of open surgery. In their meta-analysis Novara et al.[20] reported that operating times are similar between open and robotic assisted radical prostatectomy, with the latter taking around 2.5 hours. When the duration of hospital stay is evaluated, some studies have reported a shorter hospital stay of patients with the robotic procedure.[16,20-22] In contrast, in some centres that have adopted the open procedure as routine, duration of hospital stay has been shown to be similar to that of the robotic procedure.[23] However, rather than an evaluation of the differences in length of hospital stay, there is a need for extensive studies evaluating functional outcomes such as the time of return to work and physical activities of the patients.

ONCOLOGICAL RESULTS

The primary aim of treatment, which is of greater importance than morbidity following radical prostatectomy, is the oncological outcome. The two most important parameters in the oncological follow-up of radical prostatectomy are a positive surgical margin and biochemical recurrence status.

The status of a positive surgical margin after radical prostatectomy is accepted as an independent risk factor in the prediction of recurrence of the disease.[24,25] As the robotic method has no haptic feedback, some surgeons speculated that this technique could increase the rate of positive surgical margin. However, studies of the robotic method have shown that it has not increased the rate of positive surgical margin. To eliminate these uncertainties, studies have been conducted comparing the robotic method with both open and laparoscopic radical prostatectomy and the rates of positive surgical margin have been observed to be similar to the other two methods.[26-30] However, authors critical of these studies have stated that patients selected for the robotic procedure were generally low-risk prostate cancer patients and the results could be different with a high-risk group. In 2013 Yuh et al.[31] published a systematic review of 12 studies including 1,360 high-risk patients managed with robotic prostatectomy and reported an average postive margin rate of 35% (range: 12–53%).

Studies using prostate specific antigen as a marker of biochemical recurrence have demonstrated that robotic prostatectomy does not increase biochemical recurrence rate when compared to the open technique. Although there are not as many long-term follow-up studies looking at the robotic method it can still be safely used, even in high-risk patients.[32,33] Menon et al.[34] presented the robot-assisted laparoscopic radical prostatectomy results of a single-centre study with a mean 5-year follow-up of approximately 1,400 patients and biochemical recurrence rates were observed to be similar to those of open surgery at 10%.

FUNCTIONAL RESULTS

Despite the good primary target oncological results after radical prostatectomy, the postoperative functional outcomes are of great concern to the patient, particularly incontinence and potency.

Urine leakage after radical prostatectomy is a troublesome condition. Therefore, studies comparing the robotic procedure with other methods have sought to answer the question of whether it can provide better incontinence results.[17,35-38] In these studies, the mean continence rates in the robotic procedure have generally been in the range of 90–95%. In a review by Coelho et al.,[17] continence was accepted as patients using one continence pad at most per day, and at the end of 1 year the continence rates were reported as 79% in the open method, 84.8% in the laparoscopic method, and 92% in the robotic method. Some studies have compared the open, laparoscopic, and robotic techniques and have observed no statistically significant difference between them in respect of incontinence rates[39] while others have stressed that the main advantage of the robotic method over others is the improved early continence status.[37,38] Although there are some conflicting data it seems likely that robotic surgery may provide some advantage in regaining continence early compared with other methods, particularly open surgery. However, it must not be forgotten that there are other factors affecting continence, such as the patient's age, the preoperative continence status, and the experience of the surgeon.

Erectile dysfunction is another problem which could develop postoperatively following radical prostatectomy. Therefore, since the time that radical prostatectomy was accepted as the gold-standard treatment for local prostate cancer, there has been the consideration of how erectile function can be better preserved and studies have been conducted on this subject. Walsh and Donker[5] first stated that the neurovascular bundle could only be seen in the posterolateral to the prostate. However, later studies showed that with the growth of the prostate in the fetus and neonates, a different course was seen of this location with distribution towards the lateral surface.[40] Eventually, many authors reached a consensus that nerve distribution was in the 2 and 10 o'clock positions on the prostate lateral surfaces; two-thirds of the prostate lateral surface nerves were in the posterolateral and one-third were in the anterolateral surface.[40-46] It was concluded that in nerve preserving radical prostatectomy, it was necessary to make the fascia incision more anterior at the level of 2–10 rather than at the level of 4–8. In this context, in the study by Savera et al.,[43] high anterior release was applied to patients in robotic radical prostatectomy and very good results were obtained.

As use of the robotic system has the advantages of three-dimensional, high-quality, detailed imaging, it facilitates protection of the neurovascular bundle. Various studies have compared the results of robot-assisted radical prostatectomy with both open and laparoscopic methods and have obtained better potency rates with the robotic method.[26,27,32,35,47] Hakimi et al.[35] presented the bilateral nerve protection results of radical prostatectomy performed laparoscopically and with the robotic method, and at the end of 1 year the potency rates were observed to be 71% and 77%, respectively.[35] Two other studies also emphasised better 1-year potency rates from the robotic procedure compared with the laparoscopic method.[27,32] Several authors have suggested that the observed improvements in early potency are due to the ability to perform more accurate nerve-protective surgery with the robotic method.[26,48] However, in some studies which have evaluated the quality of life of patients after radical prostatectomy, no significant differences have been observed between the open and robotic method in respect of sexual functions at the end of 1 year.[49]

Similarly, in a survey by Barry et al.,[50] an extensive investigation was made into potency and continence after radical prostatectomy and no significant differences were found between open and robotic methods. In conclusion, it is unclear whether better potency results are obtained following a robotic procedure. However, in the preoperative evaluation, factors that could affect

the potency rates must be taken into consideration, such as the disease stage, the patient's age, concomitant diseases (diabetes, etc.), the experience of the surgeon, and the surgical technique (intrafascial, interfascial). Patients must be informed accordingly before the operation and the expectations must be presented by stating the risks of what could occur postoperatively. Thus, the patient can avoid disappointment in the postoperative period.

COSTS

The additional cost of the robotic procedure is without a doubt its greatest disadvantage. In a systematic review by Tandogdu et al.[51] in 2015 evaluating the economic burden of the robotic system, it was emphasised that the average costs of robotic radical prostatectomy were higher than those of both the open and laparoscopic methods. The cost ranges were stated as $7,504–$9,737, $4,931–$10,567, and $6,320–$10,991, respectively. In another study conducted in Japan comparing the robotic procedure with other methods, the robotic radical prostatectomy and open method was reported to be 53% and 13.2% more costly than the laparoscopic method, respectively.[15] In a broad-based study in the USA, it was concluded that despite the shorter hospital stay and lower complication rates of robotic radical prostatectomy compared to the open method, the total hospital costs were greater ($11,932 versus $9,390).[52] In contrast, some authors have stressed that with experienced surgeons working in centres performing high numbers of robotic radical prostatectomy, this cost difference can be reduced to a minimal level.[53,54]

CONCLUSION

The use of minimally invasive robotic radical prostatectomy continues to increase. Previous studies have reported pleasing oncological results with the use of the minimally invasive robotic radical prostatectomy even in cases of advanced prostate cancer. Although there is an additional financial burden, in comparison with open surgery radical prostatectomy, the robotic method may provide some advantages in terms of functional outcomes such as continence and potency. However, there is clearly a need for future prospective studies of large patient series with longer follow-up to further clarify the financial issues and the oncological and functional outcomes.

Another important point is that preoperative evaluation must be applied to patients (the stage of disease, comorbidities, etc.) and they must accordingly be informed in detail of what to expect postoperatively. Thus, patients will not have unrealistic expectations and will avoid the possibility of disappointment.

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RISK OF PREGNANCY IN BREASTFEEDING MOTHERS: ROLE OF THE PROGESTERONE VAGINAL RING ON BIRTH SPACING

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ABSTRACT

The progesterone vaginal ring (PVR) Progering® has been shown to be effective as a contraceptive in breastfeeding women who need safe and effective methods of spacing pregnancies. Previous clinical trials, of 1-year duration, demonstrated its efficacy to be similar to that of the intra-uterine device (IUD) during lactation. The duration of lactational amenorrhoea is significantly prolonged in PVR users in comparison with IUD users with fewer median numbers of bleeding/spotting episodes and days. This delivery system designed for 3-month use needs to be renewed every 3 months as long as breastfeeding continues, for up to 1 year. The frequency of breastfeeding, breast milk volume, and infant growth were not different in PVR or IUD users, and the safety of this new method has been well documented. This article reviews the literature and describes the mechanism of action of the PVR during lactation to bring additional protection over exclusive breastfeeding only, during the first year postpartum. Further evaluation of the PVR acceptability in different populations where breastfeeding is popular and highly recommended for the infant's benefit is warranted.

Keywords: Progesterone, lactational amenorrhoea, vaginal ring, contraception.

INTRODUCTION

The progesterone vaginal ring (PVR) Progering® is a vaginal ring which contains progesterone and can enhance the effect of breastfeeding on birth spacing.

CONTRACEPTIVE EFFECT OF EXCLUSIVE BREASTFEEDING

Postpartum family planning has received renewed focus with the issuance of new guidelines from the World Health Organization (WHO). Some estimates suggest that the unmet need for contraception among women during the postpartum period is >60% in developing countries.[1,2]

In this context, the lactational amenorrhoea method (LAM) is recognised as an effective means of postponing the return to fertility in breastfeeding mothers as indicated by the onset of menstrual bleeding.[3-9] LAM is defined as a method that can effectively protect a woman from pregnancy if she meets all of the following three criteria: 1) her period has not returned since her baby was born; 2) she is breastfeeding exclusively (fully) day and night, i.e. breast milk is the only source of water or nutrients during the first 6 months as long as the infant's growth is adequate; and 3) her baby is <6 months old. As soon as the woman no longer meets one of these criteria, pregnancy rates increase and she needs to begin using another contraceptive method.

Based on recent demographic and health surveys however, a low proportion of women report compliance with the three criteria for the use of LAM (usually <5% of breastfeeding women).[10] The results from a large multicentre study on efficacy of LAM conducted in the early 1990s suggest that deviation from specific use of each of the three criteria does not cause a significant upsurge in

pregnancy rates.[11] It has been shown that the PVR as a new method of contraception during lactation can provide additional protection to breastfeeding women who want to space their pregnancies for >1 year but may not comply with the strict criteria of LAM.

Lactational amenorrhoea and its associated infertility have been shown to contribute to birth spacing, although variable effectiveness has been reported among different communities. In a population of Chilean women highly-motivated for prolonged breastfeeding (N=236), who breastfed up to 8 times per day, the risk of experiencing the first bleeding was reduced. Of the fully breastfeeding women, with a high number of nursing episodes across day and night, 25% and 50% had started their menstrual cycle by the end of 5 and 8 months postpartum, respectively.[3] After the first postpartum menses, the risk of pregnancy for breastfeeding women increases substantially.[3,12] The cumulative probability of pregnancy changes from 0.9% in amenorrhoeic women to 36% in cycling women at 6 months postpartum, and at 12 months the pregnancy rate increases further from 17% (in amenorrhoeic women) to 55% (in cycling women).[12]

Díaz et al.[4] demonstrated that the onset of bleeding before 6 months postpartum in fully breastfeeding women predicts a higher risk of pregnancy. The investigators calculated the probability of experiencing the first bleeding and the probability of pregnancy in 236 women who were fully breastfeeding, not using contraception, and enrolled during Month 1 postpartum.[4-11] The cumulative probability of bleeding and of pregnancy was 52% and 9.4% at Day 180 postpartum, respectively. The risk of pregnancy was <2% in the subset of amenorrhoeic women.[4] These results confirmed that the LAM provides effective contraceptive protection during the first 6 months postpartum. They also suggested that the first postpartum bleeding marks a discernible increase in the risk of pregnancy.[4,12]

After Month 6 postpartum, when breastfeeding will probably cease to be 'full' or 'nearly full', it is increasingly likely that ovulation will precede the first vaginal bleed. Therefore, the protection against pregnancy that is afforded by breastfeeding decreases over time to levels lower than those of other family planning methods.[5]

Based on these data, participants in a Bellagio Consensus Conference[5] concluded that the maximum birth spacing effect of breastfeeding is achieved when a mother 'fully' or 'nearly fully' breastfeeds and remains amenorrhoeic. When these two conditions are fulfilled, breastfeeding provides >98% protection from pregnancy in the first 6 months.[5]

CONTRACEPTIVE METHODS IN BREASTFEEDING WOMEN

As a result of growing urbanisation and changing social norms about the role of women in developing countries, the duration of exclusive breastfeeding and its impact as a contraceptive strategy has been reduced. This situation has given rise to the need for a contraceptive method that could extend the infertile period following delivery, especially in countries where access to other contraceptives is limited and where a longer duration of breastfeeding is a social norm and a major benefit to infant health.

According to WHO Medical Eligibility Criteria (MEC), several suitable methods for women who are breastfeeding can be recommended.[13] Progestin-only pills have a longer half-life than progesterone but need to be taken daily at approximately the same time. Long-acting reversible contraceptives (LARCs) such as the progestin implant or an intrauterine device (IUD) require access to trained healthcare providers for insertion and removal. The PVR was developed as a new user-controlled method that delivers a natural hormone for 3 consecutive months hence not requiring daily attention by the user. As opposed to oral contraceptives taken daily or LARCs, vaginal rings designed for 3-month use are often called mid-acting delivery systems. Since progesterone in breast milk is metabolised quickly after ingestion, the steroid exposure to the infant is limited.

MECHANISM OF ACTION OF THE PROGESTERONE VAGINAL RING

The contraceptive mechanism of action of natural progesterone is similar to that of progestin-only pills, i.e. it suppresses ovulation and reinforces the prolactin response to suckling.[9] Díaz et al.[9] explored the mechanism of action of progesterone rings in lactating women by comparing ovarian function and prolactin levels between women who chose either a PVR or a copper IUD at Day 60 postpartum.

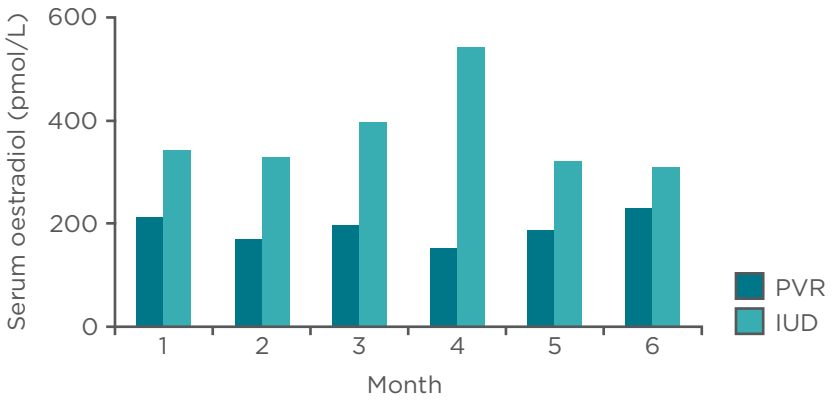

Figure 1: Mean oestradiol serum levels by month of PVR or IUD use.
Mean of the highest oestradiol level in lactating women treated with a PVR or a copper-T IUD $p<0.05$ except in Month 6.
PVR: progesterone vaginal ring; IUD: intrauterine device.
Adapted from Díaz et al.[9]

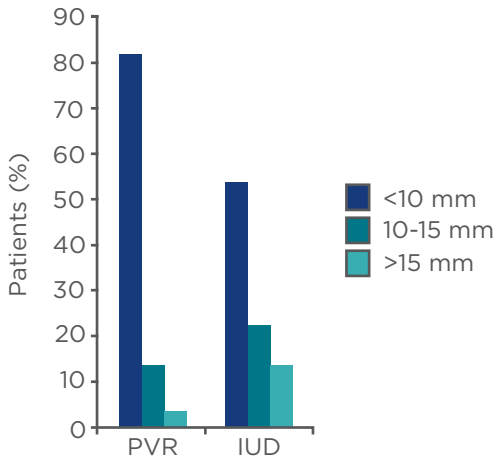

Figure 2: Follicle diameter in users of PVR or IUD from 3–8 months postpartum.
A significantly higher proportion of women (%) in the copper-T IUD group showed follicles >15 mm than those in the PVR group ($p=0.0006$; Fisher's exact test).
PVR: progesterone vaginal ring;
IUD: intrauterine device.
Adapted from Díaz et al.[9]

Data were provided based on monthly follow-ups during 1 year of use. Frequency of breastfeeding and pregnancy rates in women who were relying only on lactational infertility were collected separately for comparative purposes.[9,12]

The women (defined as fully or exclusively breastfeeding) were instructed not to give their babies any liquid or solid food or water, and to use the breast as the only source of fluids and nutrients during the first 6 months postpartum except for the administration of vitamin drops. Milk supplements were indicated only when inadequate infant growth was diagnosed. Non-dairy meals were introduced after Month 6 postpartum.

The endocrine profile was assessed during the first 8 months postpartum in a subgroup of breastfeeding women including 36 PVR-treated women and 28 IUD-users. Pre and post-suckling prolactin (PRL) levels were measured every 2 weeks; oestradiol determinations and ovarian ultrasound were performed 2-times a week. Post-suckling PRL levels were significantly higher among PVR users (n=20) compared with IUD users (n=12); $p=0.009$. In PVR users, progesterone plasma levels ranged from 10–20 nmol/L, at lower levels than in a normal luteal phase. Similarly, oestradiol levels were lower and follicular growth was arrested at earlier stages in the PVR versus the IUD groups (Figures 1 and 2).

The authors concluded that progesterone increases the sensitivity of the breast-hypothalamic-pituitary system to suckling, as shown by the higher PRL levels in women using the PVR, and reinforces the mechanism of lactational infertility.[9] They also concluded that progesterone may affect the gonadotrophin-releasing hormone discharging process independently of suckling.[9] These results therefore support the efficacy of the PVR in suppressing ovulation for a longer duration as compared with untreated women who demonstrate resumption of follicle growth and possible ovulation, even whilst fully breastfeeding.

Table 1: Contraceptive efficacy of progesterone vaginal rings in nursing women.

	PVR users	T-Cu users	Untreated women[c]
Women	246[a]	442	226
Pregnancies/WM[b]	1/2016	2/3461	50/1552
Pearl index	0.6	0.7	38.7

[a]PVR 5 mg (n=76), 10 mg (n=109), or 15 mg (n=61).
[b]PVR and T-Cu were administered at Day 60±5 postpartum and the women were followed until Month 14 postpartum. Untreated women were followed until Month 12 postpartum.
[c]The untreated group has been collected in another study[12] and used in this table in a paper by Díaz et al.[9] as a historical comparison.
WM: woman-month; T-Cu: copper-T;
PVR: progesterone vaginal ring.

CONTRACEPTIVE EFFICACY OF THE PROGESTERONE VAGINAL RING

In the study by Díaz et al.,[9] pregnancy rates at the end of the year were 0.6% in PVR users and 0.7% in IUD users. In another study that included a population of 236 breastfeeding-only women, the pregnancy rates at 1 year were 39% (Table 1).[12] In the Díaz et al.[9] study, all women in the PVR and IUD groups were amenorrhoeic at admission. By the end of postpartum Month 8, 78% of PVR users and 29% of copper-T (T-Cu) 380A IUD users remained amenorrhoeic. The PVR group experienced a significantly lower risk of bleeding (p<0.0001) than the IUD group.

Massai et al.[14] also studied the contraceptive efficacy and safety of the PVR compared with the T-Cu IUD in breastfeeding women enrolled at three Chilean clinics. A total of 285 volunteers chose to use the PVR and 262 women used the T-Cu. Ring replacement was scheduled every 3 months. Volunteers continued in the study until weaning or completing the continuous use of four PVRs over 1 year. No pregnancies occurred in 2,320 and 2,183 woman-months of exposure with the PVR and the T-Cu, respectively.

The mean duration of lactational amenorrhoea was 361±9 days in the PVR group and 198±8 days in the T-Cu group (p<0.0001). The proportion of amenorrhoeic women at 6 months postpartum was 87.4% among PVR users and 41.5% among T-Cu users (p=0.0001). These percentages were 3 and 6-fold higher in the PVR than in the T-Cu groups at Months 9 and 12, respectively.[14] The mean number of breastfeeding episodes was similar in both groups, decreasing from a mean of 10.1 episodes per day at Month 3 to a mean of 5 episodes per day at Month 14 postpartum. Infant weights were similar in both groups.[14]

In the Population Council's large comparative multicentre trial comparing 802 women using the PVR and 734 women who received a T-Cu 380A IUD, the 1-year pregnancy rate with the ring was 1.5 per 100 (431 woman-years) and 0.5 per 100 in the T-Cu 380A cohort (533 woman-years). The percentage of women who were amenorrhoeic at 6 months postpartum was 67.4% in the PVR group and 43.7% among IUD users (p=0.0001); and at Month 12, the rate of amenorrhoea remained higher in the PVR group at 46.2% versus 16.1% in the IUD group (p=0.0001). There was no difference between groups in the mean number of breastfeeding episodes per day which was around nine meals per day at initiation and six meals per day at 12 months.[15] In addition, the weight of the infants did not differ between PVR or IUD users except at 12 months; this was attributed to more supplements given in the IUD group.[15] One weakness of this study is the large inter-study centre differences. Also, neither of the above studies are randomised controlled studies.[14,15] However, in contraceptive clinical trials most of the studies are open in design and the guidelines from stringent regulatory authorities indicate that non-comparative studies are accepted.[16]

Results of clinical trials completed to date support the following conclusions regarding the role of breastfeeding and use of the PVR to promote child spacing: breastfeeding protects against pregnancy if a woman is fully breastfeeding and remains amenorrhoeic; in this case her pregnancy risk will be about 0.9% at 6 months postpartum.[12] When a first bleeding occurs before 6 months postpartum the risk of pregnancy increases to 9% and higher.[5,12] The risk of experiencing the first bleeding is reduced while fully breastfeeding with a high number of nursing episodes per day and night.[3] Using a PVR prolongs amenorrhoea in a higher proportion of women compared with women who are breastfeeding only. At 6 months, 87.4% of PVR users are amenorrhoeic versus 41.5% in IUD users.[14]

Users of a PVR show a higher suppression of ovarian follicles as compared with women using an IUD, with a majority of follicles at a diameter <10 mm (82%), while IUD users show only 54% of follicles at <10 mm. Follicles of >15 mm were seen in 4% of PVR users and 23% of IUD users.[9] In fully breastfeeding women, pregnancy rates at the end of 1 year are observed at <1% in PVR users (treated) and at 39% in breastfeeding women not using any other contraception.[9,12]

SAFETY OF THE PROGESTERONE VAGINAL RING

Breastfeeding and Infant Growth

It should be noted that in all clinical studies involving a PVR, no deleterious effects on the frequency of breastfeeding, breast milk volume, or infant growth have been observed.[14,15] The transfer of progesterone to the infants via breast milk of mothers using progestogen-only subdermal implants was evaluated by measuring urinary pregnanediol-3-glucuronide, a progesterone metabolite.[17] At 3–4 months postpartum in nine infants and at 9–12 months postpartum in seven infants, the metabolite levels were 6.3 and 15.7 ng/L, respectively, values that did not differ significantly from those in infants whose mothers were using a T-Cu 380A IUD.[17] Based on the pregnanediol-3-glucuronide levels, it was estimated that infants ingesting 800 mL of breast milk daily were receiving approximately 5 µg of progesterone from breast milk which is almost negligible when compared to the European Medicines Agency (EMA) recommended maximum intake of exogenous progesterone that should not exceed 150 µg/day.[18]

Since the progesterone levels in milk are highly correlated with the plasma progesterone levels,[18] a child taking 600 mL of breast milk from a mother wearing a PVR will ingest (~7 ng/mL×600 mL) around 4.2 µg of progesterone per day, which represents less than the maximum recommended intake of 150 µg/day.[18] Moreover, progesterone has a short half-life when given orally (3–90 minutes) and is extensively degraded after ingestion, its bioavailability being <10%.[19] Therefore, it is unlikely that this low amount of progesterone excreted in the milk can affect the infant. This has been confirmed by controlled clinical trials where infant growth was monitored for 1 year and no difference in growth and development was noted between infants of mothers using either PVR or IUD.[15]

Adverse Events Reported in Previous Studies

The most frequent adverse events among ring users that were reported in the Chilean trial[14] included vaginal complaints (e.g. vaginal discharge, non-specific vaginitis, fungal or yeast infections, trichomonal infection, and urinary discomfort), with the rate being 3.5 per 100 women-months; significantly higher as compared with a rate of 1.9 per 100 women-months reported in the IUD group. Low abdominal pain and dysmenorrhoea were more frequent in the T-Cu IUD group. In the multicentre trial,[15] while medical complaints centred on vaginal conditions were higher among PVR users (25.8% in PVR group versus 16.8% in IUD group), objective findings at clinical examinations indicated that PVR users were diagnosed with fewer genital and pelvic conditions such as cervical and adnexal disorders compared with IUD users.[14,15]

No serious adverse events have been reported in either of the studies. The reasons for discontinuation cited by the participants in these studies included complaints such as unscheduled vaginal bleeding, increased vaginal discharge, and ring expulsion. This latter finding highlights the need for proper counselling for correct insertion of the ring. In another study, no differences were found between groups in any measurement of bone density; bone density in the lumbar spine decreased in comparison to that seen in non-breastfeeding women in the first month after delivery; no differences were found among groups after weaning.[20]

RECENT AND FUTURE RESEARCH

A large 20-centre study comparing the PVR versus an IUD has recently been completed in India.[21] Its preliminary findings are comparable to those of the studies in Chile and the low failure rate of the PVR appears to have been replicated, with a higher continuation rate in some centres.[21] More recent studies have assessed the acceptability of the PVR in breastfeeding women in Sub-Saharan Africa and one of the reasons cited for accepting the ring was the autonomy it gives to the user.[22,23]

Future research will include post-marketing safety surveillance once the product is approved in other countries and introductory research within health systems will be conducted. In addition, research on the effect of product delivery by non-physicians in developing country health systems, client

preferences, and the determinants of choice of product may bring additional information useful to tailoring the method to women's needs.

Assessing the comparative cost of the PVR to other contraceptive products in developing country markets is of high relevance; an IUD offers protection from pregnancy for many years, meaning that the average annual direct cost of an IUD is $0.58, whereas hormonal contraceptives range from $7.51-7.90.[24] The PVR brings a shorter-term user-controlled option for birth spacing, with additional benefit in breastfeeding support as compared with LARCs, and no additional cost for trained health providers.

CONCLUSION

Based on the review of the literature discussed, it may be concluded that the PVR is effective in preventing an early return of follicle growth and ovulation, and preventing the return of cycling and fertility that may occur even in women who are fully breastfeeding. Data from the clinical studies confirm the efficacy, acceptability, and safety of the PVR for contraceptive use by lactating women. The PVR has been shown to be safe also for breastfed infants with no difference in growth rate as compared with infants breastfed by mothers using an IUD. The fact that it is user-controlled and contains a natural hormone contributes to its acceptability by women, especially for those unable to gain access to provider-dependent methods for various reasons. Use of the ring would help to empower more women allowing them to take control of their fertility while they continue breastfeeding. The increase in the duration of lactational amenorrhoea is also of interest for women with low haemoglobin values as it decreases blood loss; this may represent an additional health benefit of the method.

A systematic review conducted by the WHO[25] concluded that the PVR is a safe and highly effective method of contraception for use among breastfeeding women and it should be offered to women who plan to breastfeed in the context of postpartum contraceptive counselling. In addition, in the WHO MEC the PVR has been assigned a Category 1 with the recommendation that women who use the PVR must be actively breastfeeding (e.g. at least four breastfeeding episodes per day) to maintain the efficacy of the method.[13]

A recent review of unmet need among postpartum women also suggests that in contexts where breastfeeding is common, counselling women about LAM and recommending contraceptive adoption possibly from Week 4 postpartum has programmatic rationale.[26,27]

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LIPSCHÜTZ ULCERS: A LITERATURE REVIEW BASED ON 79 CASES

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ABSTRACT

Lipschütz ulcers (LU) are acute genital ulcers that occur in women. These ulcers are painful and cause enormous emotional stress to the affected person. It is also of the utmost important for the treating physician to differentiate LU from sexually transmitted ulcers like syphilis, herpes genitalis, and chancroid. The aetiology of LU is not known but recent studies have proposed that it is associated with viral infections, such as the Epstein–Barr virus (EBV) and the cytomegalovirus (CMV), as well as other bacterial infections, such as mycoplasma. Using the PubMed database, articles published between the years of 2003 and 2015 were collected. A total of 20 studies (N=79) fulfilled the inclusion criteria and were selected for analysis. All the published articles were reviewed and relevant data extracted. The age range of patients included in these studies was 17 months to 79 years old. The causative organism was unknown in 50 patients (63%), EBV in 13 patients (16%), *Mycoplasma pneumoniae* in 4 patients (5%), CMV infections in 4 patients (5%), *Mycoplasma fermentans* in 3 patients (3.7%), mumps in 1 patient (1.2%), paratyphoid fever in 1 patient (1.2%), parvovirus B19 in 1 patient (1.2%), co-infection of influenza B and adenovirus in 1 patient (1.2%), and co-infection of EBV and CMV in 1 patient (1.2%). Even though viral and bacterial infections had been linked with LU in many of the patients included in these studies, the aetiology remains unknown. Hence, more research is warranted to ascertain the aetiological factors of LU.

Keywords: Lipschütz ulcers (LU), Epstein–Barr virus (EBV), cytomegalovirus (CMV).

INTRODUCTION

In 1912, the Austrian dermatologist Benjamin Lipschütz first described acute genital ulcers in adolescent girls without any evidence of sexually transmitted infections.[1] Lipschütz ulcers (LU) is a term synonymous with a range of others, including acute genital ulcers, reactive non-sexually related acute genital ulcers, ulcus vulvae acutum, acute vulval ulcers, and primary apthous ulcers. It is an entity that presents as acute ulcers of the labia minora or majora, introitus, fourchette, and vestibule, with systemic symptoms such as fever, tonsillitis, lymphadenopathy, and diarrhoea. LU is a diagnosis of exclusion. It mimics a wide spectrum of diseases ranging from infective causes (syphilis, herpes genitalis, and chancroid), to inflammatory conditions (Behçet's disease, Crohn's disease), and also trauma. Hence, a careful review of a patient's history, meticulous clinical examination, and thorough investigations are necessary to diagnose LU.

Table 1: Diagnostic criteria for Lipshütz ulcers.

Major diagnostic criteria	
1.	Presents with a first flare of acute genital ulcer
2.	Aged <20 years
3.	Absence of sexual contact in the past 3 months
4.	Absence of immunodeficiency
5.	Acute onset of the genital ulcer and healing within 6 weeks
Minor diagnostic criteria	
1.	Single or multiple deep, painful ulcers, with a necrotic centre
2.	Bilateral 'kissing pattern'

Table 2: Synopsis of the analysed cases.

Author and year of publication	Publication type	No. of Cases	Causes
García et al.[2] 2016	Case report	1	Unknown
Horie[31] 2015	Case report	3	*Mycoplasma fermentans*
Haidari et al.[32] 2015	Case report	1	Influenza B, adenovirus
Barrett et al.[14] 2015	Case series	7	EBV
Vieira-Baptista et al.[8] 2016	Retrospective analysis	33	CMV (3 patients), *Mycoplasma pneumoniae* (3 patients), EBV (2 patients), PVB19 (1 patient), unknown (24 patients)
Delgado-García et al.[33] 2014	Case report	1	Unknown
Kinyó et al.[20] 2014	Case report	2	Unknown
Ozuguz et al.[14] 2013	Case report	1	EBV, CMV
Archel et al.[6] 2013	Case report	1	EBV
Brinca et al.[11] 2012	Case report	1	Unknown
Truchuelo et al.[34] 2012	Case report	2	Unknown
Rosman et al.[35] 2012	Case series	12	Unknown (11 patients), *Mycoplasma pneumoniae,* (1 patient)
Sárdy et al.[13] 2011	Case report	1	EBV
Chanal et al.[9] 2010	Case report	1	Mumps
Alés-Fernández et al.[7] 2010	Case report	3	Unknown
Martín et al.[10] 2008	Case report	1	CMV
Hernández-Núñez et al.[25] 2008	Case series	4	Unknown
Bhat RM, Furtado S[16] 2007	Case report	1	Unknown
Halvorsen et al.[12] 2006	Case report	2	EBV
Pelletier et al.[36] 2003	Case report	1	Paratyphoid

EBV: Epstein–Barr virus; PVB19: parvovirus B19; CMV: cytomegalovirus.

After ruling out sexually transmitted infections, inflammatory conditions, and systemic illness, the diagnosis of LU is established by five major and one of two minor criteria,[2] as shown in Table 1. Almost all of the published literature on LU is restricted to case reports and case series.[3] The aetiology of this disease remained obscure until the 1960s, when the first cues revealed the involvement of viruses or bacteria. Earlier, Lipschütz assumed that LU was caused by auto-inoculation with Döderlein's bacillus,[4] but studies have since revealed that LU is associated with viruses such as Epstein–Barr virus (EBV), cytomegalovirus (CMV), and mumps, as well as bacteria such as *salmonella*, *Mycoplasma pneumoniae*, and *Mycoplasma fermentans*. Not only do infections cause LU, it can also be caused by drugs, albeit rarely.[5] This review of the published literature was conducted to discover the different organisms associated with LU.

METHODS

Search Strategy

A review of the literature was carried out using the PubMed database to collect all articles published about LU between the years of 2003 and 2015. 'Acute genital ulcers', 'Lipschütz ulcers', and 'non-sexually acquired genital ulcers', were the search keywords used to find the relevant articles (including case reports and series, reviews, and letters to the editor) about LU. Articles published in English which pertained to LU were included in the review.

Data Extraction

Studies were selected based on their titles and abstracts. Complete articles were retrieved for detailed analysis. Studies included were individually reviewed to identify data related to age, sexual history, clinical features, histopathology, and other

relevant investigations, as well as treatment and the associated organisms.

RESULTS

Articles published in English between 2003 and 2015 were selected for the review resulting in 20 studies involving 79 cases. A synopsis of the studies included in the review are described in Table 2.

The reporting of various aspects of the cases was inconsistent in a few of the studies. For example, the demographic aspects, serology, polymerase chain reaction, blood count, and histopathological evaluation was not described in all cases.

The age of the patients was either in the second or third decade except in cases reported in the Archel et al.[6] study (17 months), the Alés-Fernández et al.[7] study (2 months), and in the Vieira-Baptista et al.[8] study (79 years). None of the patients had provided their sexual history prior to the onset of genital ulcers except for the patients in the studies by Chanal et al.,[9] Martín et al.,[10] Brinca et al.,[11] and Halvorsen et al.[12] No patient history suggestive of trauma or child sexual abuse was identified.

Clinical Manifestations

More than 90% of the patients had multiple ulcers on the labium majora and minora. One patient had ulcers involving the perineum.[12] Tonsillitis was the most common association found in patients.[6,10,12,13] Morbilliform rash was another associated finding, as specified by Halvorsen et al.[12] and bilateral parotitis was reported by Chanal et al.[9] in their patient. Hepatomegaly and polyarthralgia was reported in one patient by Sárdy et al.[13] while García et al.[2] outlined the presence of limb haematomas in their study.

Histopathological Evaluation

Skin biopsy is not recommended as a first-line investigation because in most of the patients it revealed non-specific mixed inflammatory infiltrate in dermis. However, a skin biopsy of the patients involved in the Sárdy et al.[13] study revealed neutrophilic predominant infiltrate,[13] in the Halvorsen et al.[12] study it revealed a leukocytoclastic vasculitic picture, and in the Barrett et al.[14] study it revealed a lymphocytic arteritis picture.

Serology

Testing for HIV, syphilis, herpes simplex virus (HSV), hepatitis B, and hepatitis C was negative in all of the patients. Specific serology in certain cases was carried out to identify EBV, CMV, mumps, mycoplasma, influenza B, parvovirus B19 (PVB19), and paratyphoid. The results revealed the association of the following organisms in the patients: EBV in 13 patients (16%), CMV in 4 (5%), *M. pneumoniae* in 4 (5%), *M. fermentans* in 3 (3.7%), mumps in 1 (1.2%), PVB19 in 1 (1.2%), paratyphoid in 1 (1.2%), co-infection of influenza B and adenovirus in 1 (1.2%), co-infection of EBV and CMV in 1 (1.2%), and the aetiology could not be confirmed in 50 patients (63%). Further studies are needed to elucidate these associations.

Treatment

Since LU are reactive ulcers, specific aetiological treatment is unnecessary. LU are self-limiting and generally recover spontaneously. Hence, patients in the studies were treated symptomatically with analgesics, topical steroids, and antibiotics. Daily treatment with topical steroids has been shown to heal the ulcer within a 2-week period. Patients in the Chanal et al.[9] and Ozuguz et al.[15] studies experienced a complete recovery without any treatment. Only patients in the Bhat and Furtado[16] and Sárdy et al.[13] studies were treated with systemic steroids. None of the patients had relapses at a 3-year follow-up, except for one patient reported by Healy and Thornhill[5] and another by Martín et al.[10]

DISCUSSION

This review describes and analyses the clinical presentations and the aetiological agents associated with LU for 79 reported patients from 2003–2015.

LU, or non-sexually related acute genital ulcerative disease, occurs in women. It is characterised by a rapid onset of ulcers on the labia minora or majora, introitus, or fourchette; usually multiple, and it can be associated with fever and inguinal painful lymphadenopathy. Generally, the natural course is benign, with spontaneous regression within a few weeks. The exact incidence of LU is unknown and the average age reported in a large series of patients by Farhi et al.[17] was 16.6 years.

The pathogenesis of LU still remains an enigma. It could develop from a haematogenous spread or autoinoculation, although one hypothesis suggests that it could arise from a hypersensitivity reaction to a viral or bacterial infection, leading to the deposition of immune complexes in the dermal vessels, which in turn activates the complement

system, resulting in microthrombi formation and subsequent tissue necrosis.[18,19]

Although the cause for LU has previously remained unknown, recent studies have revealed its link to an infective aetiology; mainly viral and bacterial. This review has affirmed the following organisms associated with LU: EBV, CMV, *M. pneumoniae*, *M. fermentans*, mumps, PVB19, paratyphoid, co-infection of influenza B and adenovirus, and co-infection of EBV and CMV. The unconfirmed aetiology in 50 of the patients remains unknown.

In EBV infections, apart from the pathomechanism mentioned above, ulcers could also be due to cytolysis as seen in herpes simplex infections.[20] Kinyó et al.[21] have stressed the role of local immunoglobulin (Ig)A in the development of LU in his patients who had a partial IgA deficiency. The pathogenesis of LU due to other organisms like CMV, *M. pneumoniae*, *M. fermentans*, mumps, and paratyphoid is not yet elucidated.

LU can have flu-like symptoms, such as malaise, fever, myalgia, tonsillitis, and lymphadenopathy; with single or multiple deep and painful ulcers on the vulva, and with or without oedema of the labium majora and minora.[22] LU may present with different morphological patterns; pseudo-vesicles, herpetiform ulceration, or ulcers with eschar.[3] Very rarely, patients have skin nodules similar to erythema nodosum, and experience morbilliform rashes in the trunk.[17,23] Apart from its classical clinical presentation, Török et al.[24] described three forms of LU:

- Gangrenous
- Chronic
- Military

LU has to be differentiated from other causes of genital ulcers such as HSV infection, genital aphthosis, and Behçet's disease (Table 3).

One has to be non-judgmental in their approach to patient care with acute genital ulcers. The clinical work-up starts with a sensitive sexual history, directed towards sexual activity and potential sexual abuse. Emphasis has to be given to the examination of ocular, neurologic, gastrointestinal, and genitourinary systems to rule out systemic disease such as Behçet's disease, cyclical neutropenia, and Crohn's disease. A complete oral and skin examination is always mandatory. Due to patient discomfort, speculum examination is not routinely performed.[25] Biopsy in most of the LU patients included in the review showed non-specific mixed inflammatory infiltrate in dermis, except for patients in the Barret et al.[14] and Halvorsen et al.[12] studies which revealed lymphocytic arteritis and a leukocytoclastic vasculitis picture, respectively. Hence, histopathology has limited diagnostic value in patients with LU.[26] The following laboratory investigations have been suggested for the evaluation of acute genital ulcers:

- A complete blood count to rule out anaemia, thrombocytopenia, and neutropenia
- The serum levels of iron, folate, and vitamin B12 measured
- A Gram stain to exclude chancroid;
- Polymerase chain reaction or culture to identify HSV
- Specific serology directed towards EBV, CMV, syphilis, and HIV
- Culture for *Lymphogranuloma venereum*
- A skin biopsy from the ulcer edge is advised for ulcers lasting for more than 3 or 4 weeks.[3]

Thus, diagnosis of LU is made only after excluding the other causes of vulvar ulcers: venereal and non-venereal infections, inflammatory diseases like Behçet's disease, and trauma.

The main aims of treatment are pain relief and ulcer healing. LU is a self-limiting condition, however treatment with a brief course of systemic corticosteroids (0.5 mg/kg of prednisolone for 1-2 weeks) may help to heal the ulcers.[27,28] Generally it is thought that antiviral drugs have no role in the management of LU.[29] However, Bhat and Furtado[16] reported a rapid improvement in symptoms following treatment with Azithromycin 500 mg and intramuscular betamethasone 8 mg both once daily for 3 days, metronidazole 400 mg three times daily for 5 days, followed by dapsone 8 mg once daily.

Table 3: Other causes to be ruled out before diagnosing Lipshütz ulcers.

Sexually transmitted infections	Herpes simplex virus, syphilis, *Lymphogranuloma venereum*, Chancroid, HIV
Non-sexually transmitted infections	Epstein–Barr virus, cytomegalovirus, influenza B, paratyphoid
Systemic illness	Behçet's disease, Crohn's disease, cyclic neutropenia, iron, folate, vitamin B12 deficiency
Drugs	Non-steroidal anti-inflammatory

Patients with LU require weekly follow-up until the ulcers have healed. The mean time for ulcer healing is reported as being between 16 and 21 days.[3-5] Once the ulcer has healed, yearly follow-up is needed to rule out progression to systemic diseases like inflammatory bowel disease or Behçet's disease. In two case series by Farhi et al.[17] and Huppert et al.,[23] 6% of the patients were eventually diagnosed with Behçet's disease. Recently, Carmine et al.[30] reported the recurrence of LU, twice within a 6-month period, in a 23-year-old woman.

A limitation of this study is its inclusion of published articles exclusively from the PubMed database, leading to a small sample size; therefore, a few other organisms associated with LU could have been missed. Other limitations include the fact that the majority of relevant PubMed studies that were searched for were carried out between the years of 2003 and 2015 which is why this date range was chosen for this review. This shorter date range could have resulted in a narrower breadth of experience. Also, as many of the reviewed papers did not record whether a scar was left from LU, conclusions cannot be drawn on the long-term cosmetic effects of the condition such as scarring. Lastly, there is no recurrence of LU in the majority of studies as they assessed their patient on only one occasion and did not revisit at a later date. Therefore, assumptions cannot be made about the recurrence of LU using this information.

CONCLUSION

LU, in spite of their characteristic symptoms, are often misdiagnosed. The diagnosis is made by exclusion after ruling out sexually transmitted infections, autoimmune diseases, trauma, and other causes of genital ulcerations. LU is painful and troublesome to the affected patient and mystifying to the physician who treats them. It is vital for practicing dermatologists, paediatricians, general practitioners, and gynaecologists to be aware of this rare and challenging entity. Patients and their family members should be certain that:

- LU are not sexually transmitted
- Ulcers are self-limiting and heal spontaneously

More studies are required to determine the organisms associated with LU and the relevant guidelines to confirm a diagnosis.

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MONOZYGOTIC PREGNANCIES FOLLOWING ASSISTED REPRODUCTIVE TECHNOLOGY: A REVIEW

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ABSTRACT

Introduction: Assisted reproductive technology (ART) is associated with an increased risk of monozygotic twinning. This narrative review attempts to summarise the known literature regarding the aetiology, incidence, risk factors, diagnosis, and prognosis of monozygotic twinning following ART.

Aetiology: Monozygotic twinning is caused by the splitting of the early embryo during the peri-implantation phase. According to the classical hypothesis, the timing of the split determines the chorionicity and amnionicity, however this has been questioned in recent literature.

Incidence and risk factors: The incidence of monozygotic twinning in natural conception appears to be independent of extrinsic factors such as ethnicity and age. The incidence of monozygotic twinning is increased from 0.4% of natural conceptions to around 0.9–2.24% of pregnancies following ART. The available literature supports a role of ovarian stimulation and extended culture to the blastocyst stage in increasing the risk of monozygotic twinning. The impact of maternal age and micromanipulation techniques such as assisted hatching and intra-cytoplasmic sperm injection appear to depend on the stage of the embryo being transferred leading to significant heterogeneity between studies.

Diagnosis: The gold standard for diagnosing monozygotic twinning is genetic testing but its cost precludes it from routine widespread use. Most epidemiological studies utilise statistical estimates such as Weinberg's differential rule and tailored questionnaires. Most studies from ART units have utilised transvaginal sonography for counting the number of gestational sacs or assessing the chorionicity.

Prognosis: The prognosis of twins appears to be dependent on the chorionicity and amnionicity and is largely independent of the zygosity.

Keywords: Monozygotic, monochorionic, twins, assisted reproductive technology (ART).

INTRODUCTION

Assisted reproductive technology (ART) has been associated with an epidemic of multiple pregnancies.[1] Increasing awareness of the risks posed by higher order multiple pregnancies has led to recommendations and legislation limiting the number of embryos being transferred.[2] The availability of efficient cryopreservation techniques has also obviated the need to transfer multiple embryos in a fresh cycle by enabling cryopreservation of supernumerary good quality embryos which could be sequentially transferred in frozen cycles.[3] This sequential approach gives equivalent cumulative pregnancy rates whilst minimising the risk of multiple pregnancy.[4]

Most twin pregnancies following ART are dizygotic, yet monozygotic twins account for 0.9–2.24% of pregnancies following ART.[5,6] Monozygotic twinning in ART is increasingly coming under focus due to two factors: 1) adoption of a single embryo transfer policy has resulted in a steady decline in the number of dizygotic twins and hence monozygotic twins will increase in their relative proportion; 2) increasing patient awareness of the risks of

multiple pregnancy leads to the diagnosis of a monozygotic twin pregnancy following a single embryo transfer coming as a shock to the patient. Hence healthcare professionals need to be equipped with up-to-date information regarding the aetiology, risk factors, diagnosis, and prognosis of monozygotic twin pregnancies in ART to allow them to offer tailored evidence-based management. This narrative review summarises the known literature regarding the aetiology, incidence, risk factors, diagnosis, and prognosis of monozygotic twinning following ART.

AETIOLOGY

Twins can either be dizygotic (developing from two different eggs fertilised by two different sperms) or monozygotic (formed by splitting of a single fertilised egg). Dizygotic twins, also known as 'fraternal' twins, have different genetic compositions just like any other sibling pair. Monozygotic twins, also known as identical twins, share the same genetic composition. In natural conceptions, dizygotic twins are much more common than monozygotic twins. In addition, the rate of dizygotic twinning appears to vary according to extrinsic factors such as increasing maternal age, ethnic variation, and previous obstetric or family history of twinning. In contrast, the rate of monozygotic twinning through natural conception appears to be constant across ethnic groups and different age groups.[7]

Monozygotic twinning is caused by splitting of the early embryo during the peri-implantation period, but the mechanisms that operate to cause this split are yet to be clarified. Various theories include splitting of the inner cell mass, repeated cycles of blastocoel collapse and re-expansion, and alterations in the zona pellucida leading to abnormal hatching have been postulated.

Transfer of an embryo with a split inner cell mass has been noted to result in monozygotic twinning in a case report.[8] In the above case, two blastocysts were transferred: one with a normal inner cell mass and other with a double inner cell mass resulting in a trichorionic, triamniotic gestation. Since the trophectoderm was apparently single at the time of transfer, the authors speculated that the splitting of the chorion indicated that either the trophectoderm cells were destined to split after transfer or the splitting could not be visualised at the time of transfer. Repeated cycles of blastocoel collapse and re-expansion has been speculated to lead to partial adherence of the inner cell mass to the opposing trophectoderm leading to splitting of the inner cell mass. This has been noted using time-lapse photography.[9] A study in murine embryos has shown that *in vitro* culture could predispose to the splitting of the inner cell mass.[10] Alterations in the zona pellucida could occur due to extended culture[11] and micromanipulation techniques such as intra-cytoplasmic sperm injection (ICSI) and assisted hatching.[12] This can lead to abnormal hatching and consequently to partial or complete pinching off of a section of the inner cell mass with or without the trophectoderm, leading to monozygotic twinning.

The classical theory holds that chorionicity and amnionicity of monozygotic twins are based on the timing of the split.[13] A split prior to 3 days causes dichorionic diamniotic twinning, between 3 and 8 days causes monochorionic diamniotic twins, between 8 and 13 days causes monochorionic monoamniotic twins, and splitting beyond Day 13 leads to conjoined twins.

Table 1: Complication profile with the different types of twins.

Zygosity	Dizygotic	Monozygotic			
Timing of split (classical theory)	N/A	<3 days	3–8 days	8–13 days	>13 days
Characteristics	Dichorionic diamniotic	Dichorionic diamniotic	Monochorionic diamniotic	Monochorionic monoamniotic	Conjoined twins
Risks	INCREASING RISK OF PREMATURITY (SPONTANEOUS AND INDUCED)		TWIN-TO-TWIN TRANSFUSION SYNDROME	CORD ENTANGLEMENT	SHARING OF MAJOR ORGANS

The risk of obstetric complications also increases along the timing of the split, with dichorionic twins having the lowest and conjoined twins having the highest risk of complications (summarised in Figure 1). However, this long-held theory has now come under question, with evidence from ART centres that splitting at the morula or the blastocyst stage can lead to dichorionic twinning.[14,15]

INCIDENCE AND RISK FACTORS

The incidence of monozygotic twin pregnancies following natural conception is around 0.4%.[16] In ART conceptions, the incidence of monozygotic twinning appears to be increased with quoted figures in studies ranging from 0.9–2.24%.[5,6] This increased risk appears to be mediated by a multitude of factors which have been reviewed in the literature.[5,6,17]

Maternal Age

Monozygotic twinning rates in natural conception appear to be largely independent of extrinsic factors such as maternal age.[7] However, the effect of maternal age on monozygotic twinning in ART has been controversial, with some studies quoting an increased risk in women >35 years of age,[18,19] some quoting a reduced risk in older women,[17,20] and others showing no significant difference with age.[12] A recent large retrospective cohort study by Kanter et al.[6] in 2015 appeared to suggest that there may be a differential impact of maternal age with respect to the day of embryo transfer. In this study, there was a significant increase in monozygotic twinning rate in cleavage stage transfers if the maternal age was <30 years and a significant decrease in monozygotic twinning rate with blastocyst transfers if the maternal age was >35 years.[6] The underlying mechanism of this opposite impact of age on cleavage stage and blastocyst stage transfers remains unknown. Donor oocytes also appear to be a risk factor for monozygotic twinning but this could be secondary to a confounding effect of age.[20]

Ovarian Stimulation

Ovarian stimulation could cause hardening of the zona pellucida and other alterations in the embryonic development which would predispose to monozygotic twinning.[21] This is supported by data showing an increased risk of zygotic splitting in the East Flanders Prospective Twin Study following the use of clomiphene citrate.[22] A similar effect has been hypothesised following gonadotropin stimulation during ART cycles.[23]

Micromanipulation

Micromanipulation techniques such as ICSI, assisted hatching, and pre-implantation genetic diagnosis cause a breach in the integrity of the zona pellucida which can lead to abnormal hatching of the embryo. This abnormal hatching could lead to bisection of the inner cell mass with or without the trophectoderm leading to monozygotic splitting. Retrospective studies evaluating whether micromanipulation techniques cause an increase in monozygotic twinning are conflicting with some supporting an association[12,18,24-28] and others refuting it.[11,23,29-34] Lending further credence to the impact of micromanipulation on monozygotic twinning is a case report where abnormal herniation has been observed *in vitro* to result in premature splitting of the inner cell mass and trophectoderm, creating two half blastocysts and leading to a monozygotic pregnancy.[35] A Cochrane review in 2012[36] has suggested a non-significant increase in monozygotic twinning with assisted hatching from 0-0.8%.[36] However, only 6 out of 31 trials in this review reported on monozygotic twinning. In addition, the rates of monozygotic twinning reported by these trials are quite low compared with large-scale retrospective studies which suggests an under-reporting of this diagnosis. The same review also reported a significant increase in multiple pregnancy rates with assisted hatching (odds ratio: 1.38, 95% confidence interval: 1.11–1.70) including data from 14 trials and the authors of the review have suggested that this could be partly attributed to an increase in monozygotic twinning. A subsequent randomised controlled trial involving 160 vitrified-warmed blastocyst cycles by Ren et al.[37] in 2013 which compared assisted hatching near the site of the inner cell mass and opposite the inner cell mass did not show any change with monozygotic twinning rates (3.9% versus 5.6%). One large retrospective cohort study suggests that the impact of assisted hatching on increasing monozygotic twinning rates might primarily be on cleavage stage embryo transfer and not on blastocyst transfers.[6] This would make physiological sense as a zona breach at the Day 2–3 stage would be a greater deviation from the natural *in vivo* state, whereas the zona pellucida undergoes dissolution after reaching the uterine cavity for implantation at the blastocyst stage. This would also explain the significant

heterogeneity between studies. Future studies looking at the impact of micromanipulation techniques should control for the stage of embryo transfer.

Blastocyst Culture

Extended culture to the blastocyst stage has been shown to be associated with increased monozygotic twinning in a recent meta-analysis.[38] Extended culture could lead to zona hardening[11] and sub-optimal culture conditions could lead to splitting of the inner cell mass[39] or blastocoel collapse,[9] all of which could explain the increased monozygotic twinning rates after blastocyst transfer. There is evidence from an 8-year follow-up study at an ART unit that improvement in culture techniques can reduce monozygotic twinning after blastocyst transfer.[40] There is also evidence of a possible synergistic effect between ICSI and blastocyst culture on monozygotic twinning.[12]

DIAGNOSIS

The gold standard for diagnosis of monozygotic twinning is genetic testing.[41] However, these techniques are expensive and hence in epidemiological practice, alternatives such as questionnaires and statistical methods incorporating fingerprinting and blood groups are used.[42-44] These alternative techniques cannot provide a confirmatory diagnosis in all cases. Large-scale epidemiological studies on the population prevalence of monozygotic and dizygotic twin pairs utilise Weinberg's differential rule, which is based on the premise that among dizygotic twins, the numbers of unlike-sexed twins and like-sexed twins are equal. Weinberg's rule has been validated in large scale prospective studies such as the East Flanders Prospective twin study.[45] Some studies utilise a combination of questionnaires and genetic testing to estimate the prevalence of monozygotic twinning.[17]

Most retrospective studies on monozygotic twinning following ART have relied on an ultrasound diagnosis of monochorionic placentation or when the number of gestational sacs was greater than the number of embryos transferred. Chorionicity can be diagnosed through antenatal ultrasound examination and be confirmed through postpartum placental examination. Antenatal ultrasound diagnosis of chorionicity relies on the thickness of the inter-twin membrane[46,47] and the 'twin peak' sign.[48] However, since up to one-third of monozygotic twins can be dichorionic, relying on chorionicity alone risks underestimating the prevalence of monozygotic splitting.[49] Estimating the number of gestational sacs may also underestimate monozygotic twinning unless a strict single embryo transfer policy is in place. A recent large retrospective cohort study including 28,596 elective single embryo transfers between 2003 and 2012 reported to the National ART Surveillance System calculated the incidence of monozygotic twinning based on the number of pregnancies with more than one fetal cardiac activity seen on transvaginal scanning.[6] However, a limitation of using the number of gestational sacs or fetal hearts as a surrogate marker is the possibility of a concurrent natural conception alongside a single embryo transfer. This has been noted in a recent study to account for up to one in five twin pregnancies following a single embryo transfer.[50]

PROGNOSIS

Multiple pregnancy places a greater strain on the health of both the mother and the offspring, resulting in greater maternal and neonatal morbidity. Couples undergoing fertility treatment do express a desire for twins in order to complete their family in one attempt[51] but follow-up studies indicate an increased risk of postpartum depression[52] and parenting difficulties among parents of twins conceived through ART.[53] ART twin pregnancies carry a higher risk of caesarean section, preterm delivery, and low birth weight than their naturally conceived counterparts.[54]

Dichorionic Diamniotic Twins

Chorionicity rather than zygosity appears to be a main determinant of obstetric and perinatal risks. Monozygotic twins with a dichorionic diamniotic placentation appear to have a similar risk profile as dizygotic twins.[55] In addition, ART-conceived dichorionic twins do not appear to be at increased risk of adverse obstetric and neonatal outcomes when compared with spontaneously conceived dichorionic twins.[56,57]

Monochorionic Diamniotic Twins

Unlike their dichorionic counterparts, monochorionic twins share a single placenta and are thus at risk of growth discordance due to unequal placental sharing and unequal vascular anastomoses, leading to twin-to-twin transfusion syndrome.[58,59] This leads to a higher risk of

intrauterine fetal demise, neonatal death, and discordant birth weight as noted in a large Dutch twin cohort study.[60] Data from the Southwest Thames Obstetric Research Collaborative (STORK) multiple pregnancy cohort indicate that the early fetal loss rate is significantly increased in monochorionic compared with dichorionic twins.[61] Data from the STORK cohort also indicated that a discordance between crown-rump lengths in early pregnancy was highly predictive for single fetal loss.[62] As monochorionic twins share a single placenta, the surviving co-twin of a monochorionic twin pair has an increased risk of abnormal cranial imaging and neurodevelopmental morbidity after a single fetal demise than their dichorionic counterparts.[63] A recent study compared 483 spontaneously conceived monochorionic twin pregnancies with 25 ART-conceived monochorionic twins and 320 ART-conceived dichorionic twins.[64] ART-conceived monochorionic twins had an increased risk of prematurity and very low birth weight leading to an increased neonatal mortality rate. ART-conceived monochorionic twins were also at increased risk of prematurity and low birth weight compared with ART-conceived dichorionic twins.

Monochorionic Monoamniotic Twins

Monochorionic monoamniotic gestations, in addition to sharing a placenta, also share an amniotic cavity. This increases the risk of cord entanglement and necessitates intensive surveillance.[65] An intensive regimen of ultrasound surveillance, medical amnioreduction with sulindac, and elective caesarean delivery at 32 weeks gestation has been suggested to improve the outcomes of monochorionic monoamniotic gestations.[66] A recent Cochrane review to assess the role of early delivery in improving outcomes for monoamniotic gestation failed to identify any eligible trials.[67] However, current literature based on expert opinion recommends elective delivery for monochorionic diamniotic twins between 34 and 37 weeks and monochorionic monoamniotic twins between 32 and 34 weeks gestation to reduce the risk of intrauterine fetal demise.[68] The exact prevalence of monoamniotic twinning following ART is unknown, but a few case reports suggest an association with micromanipulation procedures of the zona such as ICSI or assisted hatching.[69,70] This correlation needs to be explored in larger studies.

Conjoined Twins

Conjoined twins are very rare, with an incidence of 1 in 50,000 to 1 in 100,000 live births in natural conception.[71] Conjoined twins have a more adverse outcome than other types of monozygotic splitting and survival depends on the presence of other congenital anomalies, the extent of sharing of organ systems, and the timing of appropriate medical and surgical interventions. Due to its rarity, the exact prevalence of conjoined twins following ART is unknown, and literature is limited to case reports and reviews, most of which have shown an association with zona manipulation.[72]

Monozygotic Triplet and Quadruplet Pregnancies

Literature regarding monochorionic triplet pregnancies following ART is limited to case reports.[33,73-80] The prognosis appears to be poor unless selective fetal reduction to twins is conducted through cord ligation.[74] Only one case of monochorionic quadruplet pregnancies following ART has been reported in the literature which was managed through selective fetal reduction of two fetuses and the delivery of the surviving two fetuses at 35 weeks and 6 days gestation through caesarean section.[81]

CONCLUSION

Clinicians need to have a greater understanding of the difference in prognosis associated with monozygotic twinning following ART as they will be increasingly encountering patients with this diagnosis due to the rising use of ART to treat infertility. Although dizygotic twins are the most common type of twins following ART, the risks that dizygotic twins face cannot be extrapolated to monozygotic twins. Monozygotic twins appear to carry a poorer prognosis and the prognosis appears to be largely dependent on the type of chorionicity and amnionicity. There remains a need to further assess the risk factors for monozygotic twins, both to provide couples with an individualised risk assessment for monozygotic twinning and to identify strategies to reduce its prevalence.

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CARDIAC ARREST IN LABOUR LEADING TO PERIMORTEM CAESAREAN SECTION IN A WOMAN WITH A BODY MASS INDEX >46 WITH A SUCCESSFUL OUTCOME

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ABSTRACT

A 42-year-old primigravida was admitted to the delivery suite for induction of labour at term due to gestational diabetes and pre-eclampsia. Her booking body mass index was 46 and she had known, well-controlled asthma. Active labour was established. When she was 8–9 cm dilated, she required fetal blood sampling. At the end of the procedure, the patient had a sudden cardiac arrest. High-flow oxygen at the rate of 15 litres was started with bag and mask and immediate maternal cardiopulmonary resuscitation (CPR) was commenced. After 3 minutes of CPR, a decision was made to perform a perimortem caesarean section to aid effective resuscitation. The baby was delivered swiftly. The patient began to respond and showed signs of life. The patient was transferred to theatre for suturing. The massive postpartum haemorrhage protocol was initiated. The patient was transfused with three units of packed red blood cells, three units of fresh frozen plasma, and two units of platelets. The total blood loss was about 3.5 litres. She recovered in an intensive therapy unit. After 72 hours, her clinical assessment excluded any neurological or other ongoing morbidity. Amniotic fluid embolism was suspected as the cause for cardiac arrest. As the patient made a very quick postoperative recovery, a bronchial lavage was thought to be clinically unnecessary. The baby, initially admitted to the baby unit, was also discharged on Day 3 of life with no morbidity. Both mother and baby were completely well at a 3-month postnatal follow-up visit.

Keywords: Haemorrhage, high-risk pregnancy, maternal mortality, obesity, hypertension.

INTRODUCTION

Cardiopulmonary arrest during pregnancy is rare but presents a stressful clinical scenario as inability to provide prompt and swift resuscitation can lead to mortality of both mother and the fetus. Therefore, the outcome is very much dependent on the quick and co-ordinated response from a multidisciplinary team, including adult cardiac arrest team, anaesthesiology, medicine, obstetrics, neonatology, and sometimes cardiothoracic surgery. The incidence of cardiac arrest in ongoing pregnancy has been reported from 1 in 20,000 to 1 in 50,000.[1] There can be wide variation of the aetiological factors but an awareness of both the resuscitation of the pregnant woman and of undertaking a perimortem caesarean section within 4 minutes after cardiac arrest proves crucial for optimal fetal and maternal outcome.[2-4]

Recent literature reviews acknowledge that due to increasing awareness and more training through multidisciplinary obstetrics courses, and practicing skills in the obstetric units, the incidence of perimortem caesarean sections is increasing as part of resuscitation.[5] Cardiac arrest may be related to conditions unique to pregnancy or the aggravation of pre-pregnancy existing comorbidities. The commonly reported causes of cardiac arrest in pregnancy are pulmonary embolism, haemorrhage, sepsis, peripartum cardiomyopathy, stroke, pre-eclampsia/eclampsia, anaesthesia-related

complications, amniotic fluid embolism, myocardial infarction, and/or pre-existing cardiac disease.[6]

The initial management of cardiac arrest in pregnant women is the same as that of non-pregnant women but if the uterine fundal height is at or above the umbilicus, the uterus should be displaced to the patient's left side during resuscitation to minimise aortocaval compression or the patient should be tilted to the left lateral side.[7] It is crucial that delivery of the fetus is part of the resuscitation process.

CASE REPORT

We report a case of perimortem caesarean section delivery resulting in optimal postoperative outcomes for both mother and baby with no reported morbidities immediately or at 3-month follow-up. This 42-year-old primigravida was admitted to the delivery suite for induction of labour at term due to gestational diabetes and moderate pre-eclampsia with significant proteinuria. Her booking body mass index (BMI) was 46 and she had known, well-controlled asthma.

She was established into active labour after artificial rupture of membranes, which was later augmented by oxytocin infusion as per local protocol. During the later parts of the active phase of labour, when she was 8–9 cm dilated, her fetal cardiotocography was classified as pathological and therefore it was decided to do fetal blood sampling. At the end of the procedure, the patient started coughing and soon after the obstetric registrar who was performing the procedure noticed her developing central cyanosis. She was not responding to verbal and painful stimuli and peripheral pulses were not recordable. High-flow oxygen at the rate of 15 litres was started with bag and mask and an immediate maternal cardiopulmonary resuscitation (CPR) commenced in left lateral position. A cardiac arrest call was put out and the arrest team arrived to take over the resuscitation.

After 3 minutes of CPR, a decision was made to perform a perimortem caesarean section in the labour ward delivery room without anaesthetic to achieve effective resuscitation. The baby was delivered swiftly and handed over to the paediatric team. The CPR continued during this time and the patient started to respond and showed signs of life. Therefore, after intubation on the labour ward, the patient was transferred to theatre for suturing of the uterus and abdomen. It was found that the uterus had sustained extensions to the uterine incisions laterally during the delivery of the baby, contributing to the developing haemorrhage, which were repaired surgically. There was no involvement of the uterine arteries or ureters. The massive postpartum haemorrhage protocol was initiated. However, after observing sub-optimal response to medical management of post-partum haemorrhage, a B-Lynch uterine suture was applied, which controlled the bleeding. The patient was transfused with three units of packed red blood cells, three units of fresh frozen plasma, and two units of platelets. The total blood loss was estimated at about 3.5 litres.

She was transferred to an intensive therapy unit postoperatively and made a good recovery. She was able to be moved back to the normal postoperative care maternity ward after 72 hours. Her clinical assessment excluded any neurological or other ongoing morbidity.

Amniotic fluid embolism was suspected as the cause for the arrest. There was a plan to perform bronchial lavage to confirm the diagnosis, but the patient made a very quick postoperative recovery, so this was thought to be clinically unnecessary. The baby was initially admitted to the special care baby unit, but was discharged on Day 3 of life with no obvious morbidity. Both mother and baby were seen at a postnatal debriefing visit at the local hospital and both remained completely well at 3 months following the event.

DISCUSSION

Cardiac arrest during pregnancy carries a very high maternal and fetal mortality rate, due to the fact that after mid-trimester the gravid uterus in a supine position causes aortocaval compression. It can therefore limit the venous return to the heart, resulting in reduced cardiac output.

It is recommended that CPR be performed in left lateral tilt position in pregnant women. Pregnancy-associated changes, including tissue oedema and breast enlargement, mean cardiac output achieved via chest compressions is poor when compared with the non-pregnant population. Therefore, emptying the uterus and the resultant significant improvement in cardiac output can contribute to optimal outcome.

Perimortem caesarean section deliveries were recommended by Katz et al.[8] in 1986. Considering the incidence in pregnant women of high BMI,

medical comorbidities, and a relatively older age, performing a perimortem caesarean section is not an easy undertaking. It should be done within the first 4 minutes of starting CPR to maximise chances of positive outcome. Recent reviews of cardiac arrest management reveal that the timing of this procedure is the most important determinant of maternal survival. Delay can cause irreversible hypoxic injury to brain. A review of the past century's cases and a review of fetal physiology suggest that to obtain optimum maternal and fetal survival, caesarean delivery should be initiated within 4 minutes of maternal cardiac arrest.[2,3]

In regards to practical considerations, this is one of the more high-risk, unusual, and frightening situations on the delivery suite for an obstetrician and relevant staff. Surprisingly, this is not a part of the mandatory training in obstetrics and gynaecology. Moreover, the complications in this particular case of morbidly high BMI, a nearly fully dilated cervix, and deeply impacted fetal head made it an extremely difficult task to deliver the baby in the right time frame.

The latest data from MBRRACE-UK[2] suggests that there were eight maternal deaths between 2010 and 2012 in the UK from amniotic fluid embolism which might have been avoided if a perimortem caesarean section was undertaken within 4 minutes of initiation of CPR. Perimortem caesarean section is rarely performed within the recommended time frame leading to adverse outcomes.[9]

Recently, the need for an awareness of the timing in which a perimortem caesarean section needs to be performed has been highlighted through practical and emergency drill courses. The authors had such a refresher course a few weeks before the incident and it gave them the confidence to perform such a procedure without hesitation. We recommend that for all staff, including the emergency departments providing care for obstetric patients, perimortem caesarean section skills should be incorporated into their regular training to achieve effective resuscitation and a successful outcome in such cases.[8] Lastly, it should be noted that legal liability from the operation is minimal.

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Cervical Stitching Thread Size Could Significantly Affect Infant Outcome

SWITCHING from a thicker to a thinner thread size in cervical stitching could prevent thousands of premature births and intrauterine deaths, according to new research.

The cervical stitch procedure, often performed to prevent preterm births, is completed with one of two types of thread. The thicker thread, resembling a shoe lace, is around 5 mm thick, whereas the thinner thread, called monofilament, has just a 1 mm diameter. Researchers at Imperial College London, London, UK, have found that the thicker thread, used in the majority of cervical stitching procedures, is associated with an increased risk of complications, this is after factoring for age and overall health of the patient.


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The research team looked at 671 women who had received the cervical stitch procedure. Approximately half of the women were treated with the thin monofilament and the other half with the thicker thread. The rate of intrauterine death was measured at 5% among the women in the thinner thread group and a higher rate of 15% was found in the thicker thread group. The preterm birth rate was 17% in those with the thinner thread, and the number was again higher in the thicker thread group, at a rate of 28%; the rate in the general population is just 7%.

> "We found potentially dangerous bacteria grew more easily on the thicker thread. This may be because bacteria can latch onto the woven structure of the thick thread more easily than the smooth, thin thread."

In a follow-up investigation, 50 women were monitored at 4, 8, and 16 weeks after undergoing the cervical stitch procedure, during which each half of the group had received either the thinner or thicker thread. Women given the thicker thread experienced increased inflammation of the cervix and an increased blood flow to the region, which can cause the cervix to open prior to labour.

The team also looked at how easily bacteria could grow on the threads. "We found potentially dangerous bacteria grew more easily on the thicker thread. This may be because bacteria can latch onto the woven structure of the thick thread more easily than the smooth, thin thread," explained Dr David Macintyre, Faculty of Medicine, Imperial College London, London, UK.

The conclusions drawn from the study are that switching from the thicker to the thinner thread could prevent 170,000 premature births and 172,000 intrauterine deaths on a global basis, using a simple, established procedure. A further study of 900 patients is currently being undertaken to evaluate these implications more thoroughly.

Breastfeeding Twice as Likely After Home Births Compared with Hospital Births

THE LARGEST population study to examine the association between breastfeeding and the place of birth in low-risk pregnancies has found that breastfeeding is twice as likely to occur after planned home births compared to hospital births.

Researchers from Trinity College Dublin, Dublin, Ireland, found a strong positive relationship between planned birth at home and breastfeeding. The results of the study could be used to understand the reasons for a lower rate of breastfeeding after hospital births and also to identify changes that can be implemented to tackle the issue.

In their research, the team included >17,500 women from the UK Millennium Cohort Study and 10,500 women from the Growing Up in Ireland Study, making it the largest cohort to date in this area. The findings showed home birth to be significantly associated with breastfeeding immediately after birth, and with continued breastfeeding during the first 6 months. It also showed that home birth mothers were more likely to exclusively breastfeed for this initial 6-month period (22% versus 9% of hospital birth mothers).

> “Hopefully this research can help us learn from the home birth model and identify the changes that could be implemented in standard hospital-based perinatal care to encourage and facilitate breastfeeding.”

The researchers have suggested a number of potential reasons to explain the substantial difference between the breastfeeding rates in the two birth settings. Among them is that the non-clinical setting of home births can facilitate skin-to-skin contact between the mother and child immediately after birth. This is considered to have a positive effect on mother-infant bonding and breastfeeding initiation. Another explanation is the reduced stress of home births that might otherwise be caused in a hospital setting by interventions such as forceps and vacuum-assisted delivery. Stress during birth has previously been associated with stalled breastfeeding.

Prof Lina Zgaga, Department of Public Health and Primary Care, School of Medicine, Trinity College Dublin, Dublin, Ireland, a principal researcher on the study, highlighted the possible future implications of the research. “The key question

that this work raises is: when breastfeeding is so strongly recommended across the board by the medical profession, what causes lower rates of breastfeeding following hospital births? Hopefully this research can help us learn from the home birth model and identify the changes that could be implemented in standard hospital-based perinatal care to encourage and facilitate breastfeeding," she concluded.

Soya Supplementation Could Improve Health for Women with Polycystic Ovary Syndrome

SOYA, a common vegetarian and lactose alternative for many foods, may help to improve metabolic and cardiovascular health in women who have polycystic ovary syndrome (PCOS).

This is according to the findings of a recent study conducted by researchers at the Kashan University of Medical Sciences, Kashan, Iran. The team examined how a diet containing soya isoflavones, naturally occurring oestrogens found in the soybean plant, could help to lower the risks of serious health conditions caused by PCOS.

"Our research found that women who have PCOS may benefit from incorporating soy[a] isoflavones in their diets," explained Prof Zatollah Asemi, Department of Nutrition, Kashan University of Medical Sciences, Kashan, Iran. "In the first study to examine the connection, we found women who consumed soy[a] isoflavones regularly saw improvement in biological markers that reflect how effectively the body utilises insulin to process sugars and had reduced levels of harmful cholesterol," Prof Asemi outlined. The researchers allocated 70 women diagnosed with PCOS and aged between 18 and 40 years old into two groups, taking either 50 mg of soya isoflavones or a placebo every day for 12 weeks. Blood samples were used to observe metabolic, endocrine, and oxidative stress biomarkers at the beginning and end of the study.

> "Our findings indicate consuming soy[a] isoflavone regularly may help women with PCOS improve their metabolic and cardiovascular health."

The findings showed that when compared with the placebo group, intake of soya isoflavones significantly decreased the levels of insulin as well as other biomarkers associated with insulin resistance, a condition that can lead to Type 2 diabetes. The soya isoflavones were also found to reduce the levels of testosterone, an excess of which is a symptom of PCOS. Low-density lipoproteins and triglycerides were also significantly reduced by an intake of soya in comparison with the placebo group.

"There is growing interest in how adding soy[a] to the diet can help address metabolic syndrome and related health conditions," Prof Asemi noted. "Our findings indicate consuming soy[a] isoflavone regularly may help women with PCOS improve their metabolic and cardiovascular health."

Environmental Focus Given to Understanding Effects of Reproductive Ageing

A NEW study linking fibrosis and inflammation in the ovaries of older women could offer researchers a new focus for tackling the effects of reproductive ageing and problems with fertility in later life.

Age is a significant factor in female infertility and mothers becoming pregnant >35 years of age are more likely to suffer miscarriages and have a higher chance of producing embryos with chromosomal abnormalities. As organs age, they become more susceptible to excessive scarring. This causes the accumulation of connective tissue, also known as fibrosis, which occurs when tissue does not regenerate or heal properly.

Prof Francesca Duncan, Department of Obstetrics and Gynaecology, Feinberg School of Medicine, Northwestern University, Chicago, USA, is the lead author of a recent study that highlights the connection of reproductive ageing with fibrosis and inflammation. "The majority of reproductive ageing research is focussed on the eggs themselves and trying to understand why their number and quality deteriorate," explained Prof Duncan. "In this study we took a different angle and instead examined the changes that occur in the environment in which the eggs develop."

The researchers analysed ovarian tissue in populations of mice. The animals were either reproductively 'young' mice, deemed equivalent to the reproductive capabilities of women in their early 20s, and reproductively 'old' mice, deemed equivalent to women aged between 38 and 45 years old. The age period of 38–45 years has been associated with decreased reproductive function and egg quality in both humans and mice. The researchers found that fibrosis was present in up to 35% of the ovarian tissue in some of the reproductively 'old' mice. They also found multinucleated macrophage giant cells exclusively in the 'old' mice, cells which are associated with chronic inflammation when found in other tissues and could be the cause of tissue damage.

> "Our work establishes fibrosis and inflammation as hallmarks of the ageing ovary and lays the foundation for considering the use of anti-fibrotic and anti-inflammatory treatments to delay or counteract the impact of reproductive ageing."

Prof Duncan concluded: "Our work establishes fibrosis and inflammation as hallmarks of the ageing ovary and lays the foundation for considering the use of anti-fibrotic and anti-inflammatory treatments to delay or counteract the impact of reproductive ageing. This has wider implications for women's health because ovarian fibrosis is a key feature of polycystic ovary syndrome and is also an unintended consequence of medical interventions such as chemotherapy and radiation."


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Removal of Tonsils and Appendix Linked to Higher Rates of Pregnancy

A 15-YEAR study has found that women who have had their appendix or tonsils removed appear to have a higher chance of pregnancy. Their findings should alleviate concerns about how the surgical removal of the appendix affects fertility.

> "This scientifically challenges the myth of the effect of appendectomy on fertility. What we have to establish now is exactly why that is the case."

A co-author of the study, Mr Sami Shimi, Clinical Senior Lecturer, School of Medicine, University of Dundee, Dundee, UK, explained that young women who have had an appendectomy were previously believed to be less likely to become pregnant in later life. However, in 2012, Mr Shimi and colleagues conducted a study that found an appendectomy may have the opposite effect on fertility. "Our first study produced such a surprising result, that women who have had their appendix removed actually appeared more likely to become pregnant, that we wanted to look at a wider group to establish whether this was related to the removal of the appendix, which if left can be a cause of inflammation," explained Mr Shimi.

In the new study, the team looked at 54,675 women who had received an appendectomy, 112,607 women who had a tonsillectomy, and 10,340 women who had both surgical procedures. All of the procedures had taken place between 1987 and 2012. The pregnancy rates of the women were analysed and compared with 355,244 age-matched women from the general population in the UK who had not had either of the procedures.

The women who had received either an appendectomy, tonsillectomy, or both, were found to have higher pregnancy rates. In the general population the pregnancy rate was 43.7%, in women who had an appendectomy it was 54.4%, and in women who had undergone a tonsillectomy it was 53.4%. For those who had both surgical procedures the pregnancy rate was the highest, at 59.7%.

The researchers are keen to emphasise that their findings do not suggest that women should undergo these procedures in order to increase their chances of becoming pregnant. Instead, they hope that the results will ease young women's concerns about the effect of an appendectomy on fertility in later life. "This scientifically challenges the myth of the effect of appendectomy on fertility. What we have to establish now is exactly why that is the case," Mr Shimi emphasised. The researchers discussed the possibility of a biological cause for why the procedures appear to improve fertility, however a behavioural explanation was felt to be more plausible.


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LISA Named Best Ventilation Strategy Against Chronic Lung Disease in Preterm Infants

THE RESULTS of a comprehensive review show that Less Invasive Surfactant Administration (LISA) is the best ventilation strategy for preventing chronic lung disease in preterm infants.

LISA was determined the most effective method after researchers from McMaster University, Hamilton, Ontario, Canada, compared seven different ventilation strategies. The team based their findings on data collected from 30 different trials which involved over 5,500 infants born before Week 33 of gestation. The researchers believe that the results of this study could be important for clinicians around the world who are involved in the resuscitation and treatment management of preterm infants.

"These preterm infants have a high risk of death or severe complications, many of which come from the fact that their lungs have not yet fully developed," explained the lead authour of the study, Tetsuya Isayama, Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Ontario, Canada. "Although a number of factors contribute to long-term breathing problems, or chronic lung disease, one of the major factors is lung injury from too much pressure and expansion from the breathing machine with a large breathing tube in the windpipe. Therefore, it is important to select as gentle breathing assistance as possible for preterm infants, but until this study, the best method was not known."

The other ventilation strategies compared in the study were nasal continuous positive airway pressure (CPAP), intubation and surfactant administration followed by immediate extubation, non-invasive intermittent positive pressure ventilation, nebulised surfactant administration, surfactant administration via laryngeal mask airway, and mechanical ventilation.

LISA is a non-invasive strategy that involves giving surfactant, a substance which keeps the lung's alveoli open, to infants via a soft, thin tube placed in the windpipe while breathing is supported by a mask over the nose. The researchers found that it was most effective in preventing the primary outcome of death and/or chronic lung disease, as well as secondary outcomes including air leak in the lungs. On average, the use of LISA resulted in 164 fewer preterm babies per 1,000 dying or having long-term breathing problems when compared with those who used a breathing machine and a large breathing tube.

> "...it is important to select as gentle breathing assistance as possible for preterm infants, but until this study, the best method was not known."

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UPCOMING EVENTS

26th World Congress on Ultrasound in Obstetrics and Gynecology (ISUOG)

25th–28th September 2016

Rome, Italy

International experts will deliver lively discussions on subspeciality topics through plenary lectures, didactic workshops, and a series of masterclasses. These will include: managing ovarian masses, abnormally invasive placenta, and the Zika virus. Live-scan demonstrations will also be an important feature of this event, supported by clinical guidance on advances in ultrasound and developing technologies.

25th Annual Congress of the European Society for Gynaecological Endoscopy (EGSE)

2nd–5th October 2016

Brussels, Belgium

Tailored to a range of specialities, this congress will discuss the important successes of endoscopic procedures in recent decades. Among an array of topics, speakers will explore the continuing relevance of endoscopy within gynaecology, including future endoscopic approaches to artificial ovaries. The event will feature a Women Surgeon's Lunch chaired by Prof Liselotte Mettler and Dr Anastasia Ussia, hosting a series of lectures to discuss the impact of training in endoscopic gynaecologic surgery.

American Society for Reproductive Medicine (ASRM) Scientific Congress

15th–19th October 2016

Salt Lake City, Utah, USA

The scientific programme will provide a comprehensive exploration of the latest molecular and genetic techniques in reproductive medicine and how this area affects public health across the world. The continuing medical education portion of the congress will provide a series of plenary lectures, symposia, and interactive sessions. The topics included will range from the impact of environmental factors and stress on reproductive health, to reproductive genetics and epigenetics.

91st National Congress of the Italian Society of Obstetrics and Gynaecology (SIGO)

16th–19th October 2016

Rome, Italy

This event will draw attention to the challenges faced in continuing therapeutic innovations for the protection of women's health, alongside its organisational and cultural challenges. Roundtable discussions will explore the contemporary issues of obstetrics and gynaecology, including concerns within occupational medicine and technological innovation. The congress will be split into six areas, each dedicated to various topics of women's health.

24th World Congress on Controversies in Obstetrics, Gynecology and Infertility (COGI)

10th–13th November 2016

Amsterdam, Netherlands

The congress will provide a comprehensive overview of the latest research developments within the areas of fertility, mechanical fertility, gynaecology, and feto-maternal medicine. Distinguished gynaecologists and scientists will debate and discuss widely across the 4-day event. This will include discussion of infertility in older age, pregnancy risks for obese patients, and devising the next steps after failure with assisted reproductive technology.

Medical Complications in Pregnancy for Obstetricians, Physicians, and Obstetric Anaesthetists

16th–18th November 2016

London, UK

This annual course, now in its 22nd year, has been designed for obstetricians, physicians, and obstetric anaesthetists caring for pregnant women with medical disorders. Leading specialists will discuss important and common medical problems complicating pregnancy and they will also cover the management of disorders predating pregnancy. Topics include: complications of pregnancy, delivery, and the puerperium.

23rd Annual Scientific Meeting of the Middle East Fertility Society (MEFS)

17th–19th November 2016

Istanbul, Turkey

This event allows the MEFS to bring fertility specialists from across the Middle East region to come together and exchange medical knowledge as well as the latest scientific discoveries. The scientific programme features a wide range of speakers who will discuss a diverse selection of topics from across the field of reproductive health, exploring the areas of minimally invasive reproductive surgery, endometrial receptivity, and male infertility.

33rd Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE)

2nd–5th July 2017

Geneva, Switzerland

As seen in previous years, ESHRE 2017 will continue to provide an important forum for the advancement of our contemporary understanding of reproductive biology and medicine. The event will promote the latest research findings in human reproduction and embryology across Europe. Its scientific programme will feature sessions that explore a wide array of topics and engage with the emerging issues encountered within them. This includes the realisation of ovarian rejuvenation, the ethics of germline genome editing, and the links between daily exposure to plastic and infertility. A keynote session also includes an update on the latest advances in non-invasive prenatal testing. Industry-sponsored symposia and paramedical presentations will also be featured.

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