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"Publishing this journal has been a real labour of love and I believe the hard work, passion, and dedication of all those involved has resulted in a very special publication."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

Aims and Scope

The European Medical Journal (EMJ) is an online only, peer-reviewed, open access general journal, targeted towards readers in the medical sciences. We aim to make all our articles accessible to readers from any medical discipline.

EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

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- Peer review, which is conducted by EMJ's Peer Review Panel as well as other experts appointed due to their knowledge of a specific topic.
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This Publication

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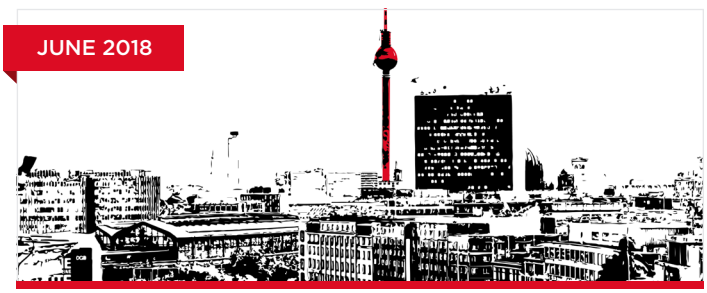
Front cover and contents photograph: Barcelona, Spain, home of the ESHRE 2018. © Brian Kinney / 123rf.com

EMJ Repro Health.



European Medical Journal 3.2

EMJ 3.2 has entered the building! This truly multidisciplinary eJournal returns for its 10th issue and its second of 2018. *EMJ 3.2* includes a plethora of high-quality peer-reviewed...

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Welcome

Welcome to this year's edition of *EMJ Reproductive Health*. Although birth rates are declining across Europe, interest in reproductive medicine is certainly not dwindling. Indeed, in today's demographically shifting society, the field has arguably taken on greater importance than ever, and we are proud that *EMJ Reproductive Health* is at the forefront of the dissemination of scientific information in this regard.

The EMJ once again attended the Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), this year held in Barcelona, Spain. The reporting team returned full of vigour, with a host of interesting stories to include in the journal's Congress Review section, including dietary considerations concerning sperm quality and progress towards artificial ovaries. To complement our coverage of the ESHRE Annual Meeting, we have included a selection of abstract reviews based on current research revealed at the event and provided by the presenters themselves. Topics include a retrospective analysis of follicle flushing during oocyte retrieval and the long-term psychological impact of interrupted fertility in cancer patients.

A quartet of interviews bring you significant insights into the minds of *EMJ Reproductive Health* Editorial Board members, covering a wide range of topics for conversation. As well as delving into current work and research, desires for the future, and opinions on the field in general, the interviewees also offer personal advice for the next generation of researchers.

The field of reproductive medicine combines cutting-edge scientific research with ethical conundrums and social considerations, and *EMJ Reproductive Health* captures this spirit within its peer-reviewed articles. For example, the Editor's Pick encapsulates how the field of medicine has changed over recent decades. Patient-focussed outcomes are increasingly taking centre stage, meaning medical professionals are now considering new priorities in the treatment and management of conditions. In this paper, Herrero and Chan outline the psychosocial need for addressing fertility concerns in male cancer patients, as well as considering perceptions and practical strategies for service improvement.

Finally, I would like to take this chance to thank all of those who contributed to the production of *EMJ Reproductive Health 4.1*. Publishing this journal has been a real labour of love and I believe the hard work, passion, and dedication of all those involved has resulted in a very special publication.

Happy reading!

Spencer Gore

Spencer Gore

Chief Executive Officer, European Medical Group

Cryogatt

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Foreword

Dear colleagues,

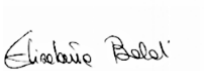
It is my pleasure to welcome you to *EMJ Reproductive Health 4.1*.

The European Society of Human Reproduction and Embryology (ESHRE) 2018 meeting was held in Barcelona, Spain, from 1st–4th July. With >12,000 participants, ESHRE 2018 reached a new record of attendance, confirming its primary role as a unique arena for scientists and operators of reproductive health to meet and exchange opinions and experiences. The programme was, as usual, exceptional, covering all the main themes of reproduction, including embryo health as well as advanced assisted reproduction technologies. Of particular interest were the sessions about male and female fertility preservation, which is fast becoming a hot topic. The session entitled 'The Aging Male' hosted two exceptional scientists, Prof John Aitken, who explored the world of oxidative stress as one of the main determinants of testicular and sperm dysfunctions with age, and Dr Jorge Gromoll, who presented interesting data about healthy aging in men, demonstrating that, in the absence of any disease, testicular function may be maintained.

The present issue of *EMJ Reproductive Health* contains a compendium of interesting peer-reviewed articles encompassing several important topics related to reproductive health. Herrero and Chan explore the important topic of 'Kids after cancer? Meeting male patient's fertility needs during cancer care' in my Editor's Pick for this edition. In this article, the authors clearly evidence the need to provide young and adult male cancer patients with appropriate counselling about their future fertility perspectives and give them the possibility to bank semen samples. There is evidence that men who bank their sperm feel more reassured about their future reproductive health and this helps a lot of patients face the psychological stress associated with their disease. Interestingly, the article considers the point of view of all the stakeholders (patients, their parents, cancer survivors, and oncologists), evidencing the barriers that hamper the possibility of preserving semen in some cases. Among these, the cost of the procedure is probably one of the most important, highlighted by the fact that after full financial coverage was offered in the province of Quebec, Canada, the number of young and adult cancer men who cryopreserved their semen increased significantly. Similar considerations apply for young women with cancer.

I am confident that you will enjoy reading this latest eJournal. The content is incredibly interesting and stimulating!

Kind regards,



Elisabetta Baldi

University of Florence, Italy

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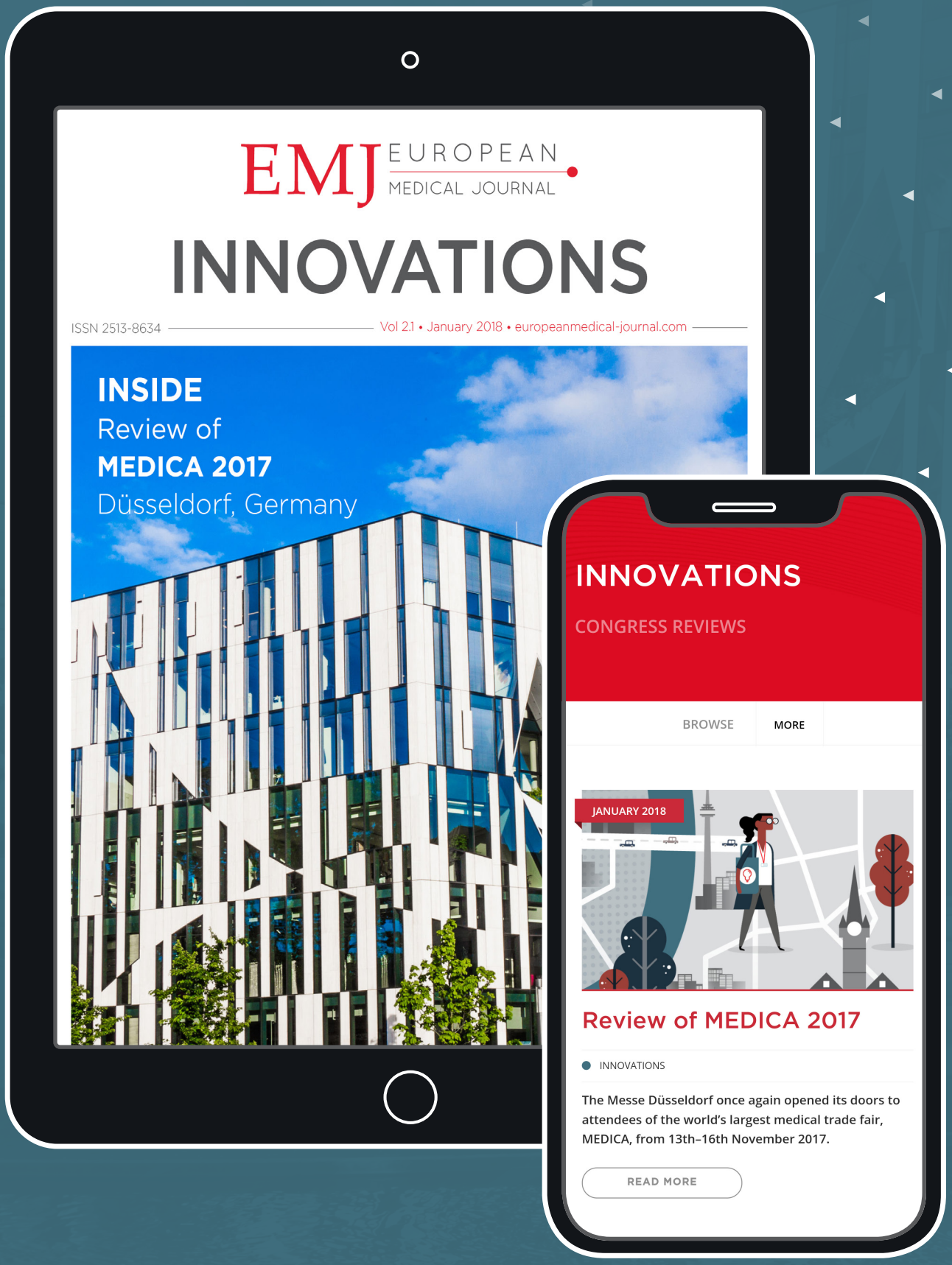

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+ Review of MEDICA 2017 Düsseldorf, Germany, 13th–16th November 2017

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+ A Transition from Disease-Centred to Goal-Directed Individualised Care of Patients with Multiple Morbidities: A Journey to Goal-Orientated Patient Healthcare Katarzyna Rygiel

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Congress Review

34th Annual Meeting of the European Society of Human Reproduction and Embryology

Location: Barcelona, Spain – Barcelona International Convention Centre

Date: 01.07.18–04.07.18

Citation: EMJ Repro Health. 2018;4[1]:12-28. Congress Review.

Beautiful Barcelona, Spain played host to the Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), with the historic city opening her doors to thousands of developmental, reproductive, and embryology specialists. Welcome to the *EMJ Reproductive Health* review of the 34th Annual Meeting of Europe's flagship reproductive health and embryology society.

The ESHRE Annual Meeting returned to Spain for a fourth time in 2018 and, as Casa Vicens glistened in the Iberian summer sunshine, Barcelona proved to be a perfect host. The meeting was held at the Barcelona International Convention Centre, located right on the seafront, and there was audible excitement in the air as 12,179 human reproduction and embryology specialists, young and old, from 130 different countries, prepared for Europe's premier celebration of research and developments within the field.

The spires of Gaudi's 136-year-old Basílica i Temple Expiatori de la Sagrada Família dominate the Catalan skyline. Famously unfinished, the church is due for completion in 2026. The ESHRE scientific programme, however, is in stark contrast to Gaudi's masterpiece. Far from incomplete, the event's comprehensive programme, masterminded by the organising committee, covered a broad range of reproductive health and embryology hot topics. Speaking before the congress, Dr Rita Vassena, head of the local organising committee, highlighted that the 77 sessions on offer during the course of ESHRE 2018's specially designed programme "cover topics of huge current interest."

Forty years ago, the face of reproductive medicine was dramatically changed forever following the birth of Louise Brown, the world's first 'test tube baby'. It was therefore fitting for Louise, the literal poster-child of assisted reproductive technology (ART), to be in attendance at ESHRE 2018, where the long-term follow-up of children born with

the help of ART was placed under the microscope. The outcomes of *in vitro* fertilisation were not the only key theme throughout ESHRE 2018; intracytoplasmic sperm injection, the topic of the Human Reproduction Keynote Lecture, and the efficacy of *in vitro* fertilisation add-on therapies were also discussed at length. Alongside the focus on the use and outcomes of ART embryo implantation failure, the modelling of the post implantation stages of embryo development *in vitro* and preconception genetic testing were both topics of hot debate following the fascinating plenary sessions and poster presentations. ESHRE 2018 broke all previous records with around 1,900 abstracts submitted to the organising committee to be considered for presentation, of which 800 were presented as posters during the 4-day event. Two hundred of the abstracts were presented as special oral presentations, a hand-picked selection of which can be found within the Abstract Review section of this Congress Review. Penned by the researchers themselves, these summaries give a fascinating insight into the latest developments on show at the enormous congress.

"ESHRE 2018 broke all previous records with around 1,900 abstracts submitted to the organising committee to be considered for presentation, of which 800 were presented as posters during the 4-day event."

Social media is an ever-growing platform that can be utilised for communication and debate; recognising this, ESHRE invited five young reproduction and embryology specialists to take over 'theESHRE5' Twitter account. Coming from a range of different backgrounds and specialities, Drs Akemini Umana, Anisha Uberoi, Beatriz Rodriguez Alonso, Michael Rimmer, and Swati Mishra posted their experiences and opinions throughout the course of the congress to great success, reaching not only the delegates in Barcelona but also those that were not able to visit ESHRE this year.

In and amongst the universal celebration of reproduction and embryology research and developments, for a select few there was even more reason to cheer as the annual ESHRE awards were presented. The Basic Science Award for oral presentation was given to Kelle Moley (USA) for her work titled "Pronuclear transfer in zygotes from diet-induced obese mice suggests a cytoplasmic origin of transgenerational transmission of mitochondrial dysfunction leading to cardiac dysfunction in offspring." New Zealand's Sarah Lensen was presented with the Clinical Science Award for oral presentation for her work on endothelial scratching during Pipelle® biopsy in *in vitro* fertilisation (IVF). European researchers were awarded the Basic Science Award for poster presentation and the Clinical Science Award for poster presentation, received by C. Alexandri (Belgium) and Asa Magnusson (Sweden), respectively. The Nurses Award was presented to Morine Cebert (USA) for her work titled: "Facilitators and barriers affecting help seeking of infertile women in the United States: A systematic review".

The FC Barcelona museum is one of the city's most popular attractions, welcoming thousands of visitors a day, but for 4 days in July 2018 the Barcelona International Convention Centre stripped the museum of its title as a record-breaking number of delegates arrived to take advantage of the "welcome chance to network with friends and meet colleagues from around the world", as described by Dr Vassena. For those of you not able to attend this year's ESHRE Annual Meeting, we are sure this captivating Congress Review will capture the spirit of the event highlighting the key research breakthroughs and summarising the best ESHRE 2018 had to offer.

The Hofburg palace is in danger of being outshone next year as ESHRE 2019 heads to the Austrian capital, Vienna. The EMJ team will be in attendance at the Imperial City to capture all the highlights and revel in the latest reproduction and embryology research and updates.

Progress Towards Artificial Ovaries

ARTIFICAL ovaries have been brought one step closer to reality as a result of work undertaken by researchers from the Rigshospitalet, Copenhagen, Denmark. The groundbreaking nature of the research was highlighted by Dr Susanne Pors, Rigshospitalet, who declared: "This is the first time that isolated human follicles have survived in a decellularised human scaffold." The details of this work were reported in a ESHRE press release dated 2nd July 2018.

Although fertility perseveration treatment via ovarian tissue freezing already takes place, for instance, ahead of radiotherapy or chemotherapy, under the current methodology there is a risk, although slight, of reintroducing the original malignancy when regrafting the original cryopreserved tissue. Dr Pors explained a way to overcome this issue: "A bioengineered ovary would allow the growth and development of reseeded frozen-thawed early stage follicles in a tissue bed which is free of malignancies."

"This is the first time that isolated human follicles have survived in a decellularised human scaffold."

With this solution in mind, the researchers extracted ovarian tissue from women who were undergoing fertility preservation treatment ahead of cancer therapy. Subsequently, the cells were put through a 3-day chemical process to eliminate the inhabiting cells of the tissue and leave an extracellular matrix scaffold of the original tissue. Following this process, the researchers used DNA and collagen quantification to test decellularisation, which demonstrated the tissues had been completely decellularised. Dr Pors then explained the next stage: "We then found that ovarian cells and early-stage follicles were able to recellularise the decellularised tissue *in vitro* by successfully repopulating and migrating into the scaffold." Additionally, the researchers conducted transplantation experiments, using mice, which found survival and growth of early-stage follicles was possible on the decellularised matrix. The researchers noted that future studies are planned to develop this technique further by investigating how best to evaluate follicle quality and how to optimise the procedure.

Prostate Cancer Risk in ICSI-Treated Fathers

SUBFERTILE men could be at a higher risk of prostate cancer, particularly early-onset disease, after treatment with intracytoplasmic sperm injection (ICSI). According to the results of a large registry study presented at ESHRE 2018 and reported in a ESHRE press release dated 3rd July 2018, the incidence of rare and aggressive early-onset prostate cancer was higher for fathers who conceived using ICSI compared to natural conception or *in vitro* fertilisation (IVF).

Therefore, while ICSI is not a cause of or risk factor for prostate cancer, it could be considered a predictor for the malignancy, allowing close monitoring and early cancer treatment of these at-risk patients.

The Swedish registry study identified approximately 1.2 million fathers with a firstborn child in Sweden between 1994 and 2014, from whom 3,211 prostate cancer patients were selected for data analysis. Using males who conceived naturally as controls, the results showed that ICSI treatment to aid conception was associated with a statistically significant increased risk of prostate cancer of 47%; this risk was particularly relevant for patients diagnosed at <50 years old, for whom the incidence of prostate cancer was 3-times that of controls (3 patients per 1,000 versus 1 patient per 1,000, respectively). However, the risk of late-onset prostate cancer was not increased following ICSI treatment.

While these results may be alarming, the researchers stressed that the increased prostate cancer risk in the study population was related to the fertility status of the males and not the ICSI procedure, which has no biological impact on the body. Since the risk was higher in infertile men than fertile men, the team hypothesised that this may have been due to a preclinical latent tumour present at the time of ICSI administration, low levels of testosterone, or testosterone supplementation.

Other biases were also taken into consideration to explain these results, such as the increased rate of prostate-specific antigen tests in subfertile men who are candidates for more general health checks, thereby increasing the chance of detecting prostate cancer, and the study's specific location. For example, since ICSI in Sweden is only indicated in male factor infertility cases, compared to non-male infertility cases in other countries, the fathers identified from the Swedish registries generally had very poor semen quality, which may instead be the causative risk factor. Therefore, while ICSI is not a cause of or risk factor for prostate cancer, it could be considered a predictor for the malignancy, allowing close monitoring and early cancer treatment of these at-risk patients.

Real-Life Data on Social Egg Freezers Returning for Fertility Treatment

ONLY 7.6% of women who elect to freeze their eggs as an insurance policy against an age-induced decline in fertility, so called 'social freezers', return to thaw and fertilise their eggs. According to a ESHRE press release dated 4th July 2018, data from one of Europe's largest fertility centres presents real-life results on the number of women returning to attempt pregnancy and the success rate of this procedure. Study investigator Dr Michel De Vos, Centre for Reproductive Medicine, University Hospital Brussels, Brussels, Belgium, validated the importance of this study because so "little is known about these social freezers and their reproductive outcomes."

...these results begin to paint an important picture of the real-life outcomes for social freezers...

The study collected data from 563 women who froze their eggs between January 2009 and November 2017. A total of 902 assisted reproduction treatments were carried out to collect eggs, with an average of 8.5 eggs collected and frozen per patient. The mean age of women freezing their eggs was 36.5 years.

Results revealed that only 7.6% (n=43) of women who had their eggs frozen retuned to the clinic and had their eggs thawed, fertilised, and transferred. The mean age of women returning to the clinic was 42 years. Of the returning 7.6%, 43.0% used donor sperm to fertilise their eggs, indicating that the majority of those who returned had found a partner to start a family with. Fourteen of the 43 returning women (32.6%) achieved an ongoing pregnancy after embryo transfer.

With an increasing number of social freezers hoping to combat the anticipated age-related fertility decline, these results begin to paint an important picture of the real-life outcomes for social freezers who return for fertilisation treatment. Dr De Vos did comment, however, that he was unable to deduce "whether their previous decision to undergo oocyte cryopreservation has enhanced the probability of a live birth." As such, it is hoped that future studies will be able to elucidate the efficacy of freezing eggs before a decline in women's fertility on future successful live births.


Barcelona International Convention Centre
Venue of the ESHRE 2018 Congress

New Data for Immunological Treatment for Pregnancy Loss

UNEXPLAINED miscarriages are often attributed to an immune response whereby the uterus erroneously rejects the embryo or fetus. This theory has led to the development of a number of immunomodulatory treatments, many of which are not evidence-based, including the use of recombinant human granulocyte-colony stimulating factor (rhG-CSF), a licensed cancer medication. Now, as reported in a press release from the ESHRE Annual Meeting 2018 in Barcelona, Spain, a large randomised placebo-controlled study, the RESPONSE trial, has shown this treatment to be of no benefit.

"Some studies have suggested statistically significant improvements in clinical pregnancy rates, but we here have high quality evidence that rhG-CSF is not an effective treatment for patients with unexplained recurrent miscarriages."

Despite its widespread use, the evidence in favour of rhG-CSF treatment for women with recurrent pregnancy loss is limited to one single-centre randomised trial and four observational studies, spurring researchers from Tommy's National Centre for Miscarriage Research at the University of Birmingham, Birmingham, UK, and the University of Iowa Hospital and Clinics, Iowa City, Iowa, USA to conduct the present study.

During this study, performed at 21 hospitals in the UK, 150 women were randomised to rhG-CSF treatment (n=76) or placebo (n=74). These women all had ≥3 unexplained miscarriages, were aged 18–37 years, and were aiming to conceive naturally. After 20 weeks, 59.2% of women in the rhG-CSF group and 64.9% in the placebo group had achieved a pregnancy, indicating that the treatment was ineffective; live birth rates were similar in both groups.

"Some studies have suggested statistically significant improvements in clinical pregnancy rates, but we here have high quality evidence that rhG-CSF is not an effective treatment for patients with unexplained recurrent miscarriages," explained Prof Abey Eapen, University of Iowa Hospital and Clinics.

Reproductive immunology is still a "relatively new" branch of reproductive medicine and treatments derived from this field are mostly experimental, explained Prof Eapen. The RESPONSE trial results, therefore, represent key data in assessing the efficacy of this approach and guiding future research.

An Update on *In Vitro* Fertilisation

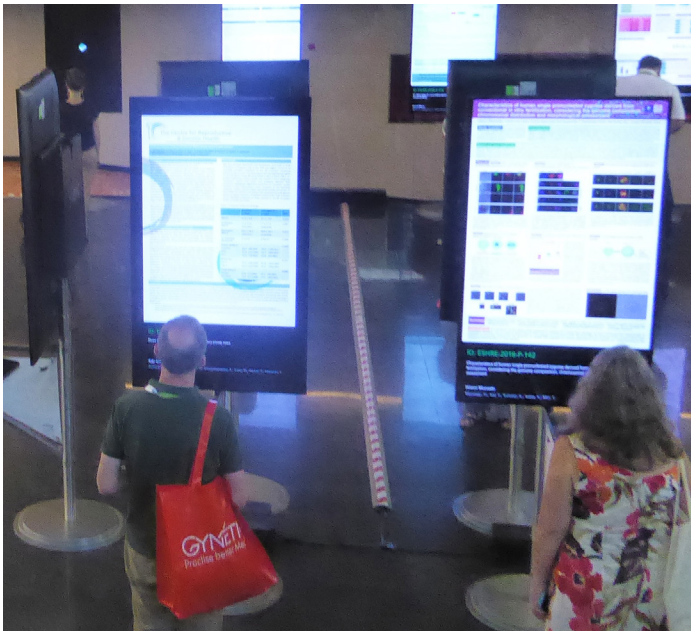
LOUISE BROWN, the first baby born using the pioneering science of *in vitro* fertilisation (IVF), is now 40 years old. Since this first procedural success in the 1970s, the use of IVF and other assisted reproductive technologies (ART) has exploded. A recent report by the International Committee Monitoring ART (ICMART), reported in a ESHRE press release dated 3rd July 2018, highlighted that >8 million children have been successfully delivered as a result of IVF since 1978.

The ICMART report collected data from regional centres across Europe from 1991–2014 and came to a number of conclusions. The data showed that there was a steep increase in the use of ART therapies during the 23-year time period and that >500,000 babies are born each year as a result of >2 million IVF and intracytoplasmic sperm injection (ICSI) treatment cycles.

...>8 million children have been successfully delivered as a result of IVF since 1978.

Developed in the 1990s, ICSI was originally intended as a strategy to combat male infertility, the ICMART report revealed that European centres continue to favour ICSI over IVF, performing 356,351 and 131,221 treatment cycles, respectively; this treatment modality was initially reserved for cases of male infertility, but it is now also used in non-male cases. Furthermore, it was identified that Spain was the European nation most active in the use of ART (119,875 treatment cycles), closely followed by Russia (110,723 cycles), Germany (96,512 cycles), and France (93,918 cycles), while the UK performs approximately 60,000 cycles annually.

While the number of IVF twin pregnancies has declined to approximately 14%, the number of single embryo transfers has risen from 11% in 1997 to 38% in 2015, with success rates stabilising at around 36% for both IVF and ICSI. The statistics generated by the ICMART appears to show an ever-improving success rate attributed to ART procedures; however, Dr Christian De Geyter, ESHRE, IVF Monitoring Programme Committee Chairman, noted that the availability of ART treatments is inconsistent, with the Danish and Belgian governments offering considerably more treatment cycles than their Italian and Austrian counterparts.



Nuts in the Diet Affect Quality of Sperm

DIET has been suggested to have an influence on the quality and function of human sperm. More specifically, the results of a randomised trial have shown that including nuts as a component of a regular diet improved the quality and function of human sperm. These results are in the context of a general decline in human sperm quantity and quality. This study was reported in a ESHRE press release dated 4th July 2018.

The results of a meta-analysis showed a "significant decline in sperm counts between 1973 and 2011", with a 1.4% annual decline in sperm concentration and a 1.6% annual decline in total sperm count.[1] Therefore, any possible solutions to reverse this trend are of importance. To test the impact of nuts on sperm quality and function, the study investigators conducted a randomised clinical trial across a 14-week period. The study cohort comprised 119 healthy men who were aged 18–35 years old. These men were divided into two groups: one group supplemented their typical Western-style diet with 60 g of mixed almonds, hazelnuts, and walnuts per day, and the second group (the control group) continued eating their typical Western-style diet without additional supplementation.

The men in the nut supplementation group presented with improvements in the parameters associated with male fertility measured by the researchers. Compared to the control group, the nut supplementation group displayed an improvement of approximately 16% in sperm count, 6% in sperm motility, 4% in sperm vitality, and 1% in morphology. Furthermore, the men eating a diet supplemented with nuts also presented with a marked decline in levels of sperm DNA fragmentation, which is a parameter strongly associated with male infertility.

"But evidence is accumulating in the literature that health lifestyle changes such as following a healthy dietary pattern might help conception..."

The presenter of the study results, Dr Albert Salas-Huetos, Andrology and IVF Lab, University of Utah, Salt Lake City, Utah, USA, sounded a note of caution that these results could not be extrapolated to the general population, as the study participants were all healthy and ostensibly fertile. However, he commented: "But evidence is accumulating in the literature that health lifestyle changes such as following a healthy dietary pattern might help conception, and, of course, nuts are a key component of a Mediterranean healthy diet."

References

1. Levine H. Temporal trends in sperm count: A systematic review and meta-regression analysis. Hum Reprod Update. 2017;23(6):646-59.

Relationship Instability Behind Majority of Egg Freezing Cases

RELATIONSHIP problems were found to be the principal reason for women freezing their eggs, according to a ESHRE press release dated 2nd July 2018. The study conducted in-depth interviews with 150 women from the USA (n=114) and Israel (n=36) who had elected to freeze their eggs for social reasons.

Interview answers were quantitively analysed and showed that 85% were without partners at the time of egg freezing as a result of being single, divorced or going through a divorce, separated, working overseas, a single mother, and career planning. Planning for a career was the least common answer, even for those who worked for a company with egg freezing insurance. Women with partners (15%) answered with four different circumstances: they were in a relationship with a man who was not ready to have children, in a relationship that was new or uncertain, with a partner who refused to have children, or with a partner who had multiple partners.

Summarising the results, Prof Marcia Inhorn, Yale University, New Haven, Connecticut, USA, explained that "Most of the women had already pursued and completed their educational and career goals but by their late 30s had been unable to find a lasting reproductive relationship with a stable partner. This is why they turned to egg freezing."

With elective egg freezing being the fastest growing services in many fertility clinics, and egg freezing cycles predicted to increase from 5,000 in 2013 to 76,000 in 2018, Prof Inhorn emphasised the importance of clinics being mindful of why women are freezing their eggs, irrespective of career choice: "Clinicians must be aware of the role that partnership 'troubles' play in the lives of egg freezing patients and make patient-centred care for single women a high priority."

"Clinicians must be aware of the role that partnership 'troubles' play in the lives of egg freezing patients and make patient-centred care for single women a high priority."

Energising Eggs Does Not Increase Pregnancy Rate

ENERGISING eggs with egg precursor cell mitochondria offers no improvement in the quality of assisted reproductive techniques. Presented in a ESHRE press release dated 3rd July 2018, results of the use of this controversial technique from a large Spanish study showed no increase in the success rate of pregnancy or live births, conflicting with positive results from previous studies.

From these results it is clear that the technique of energising eggs still has a way to go before it can be used to improve egg quality with certainty.

As a technique that has attracted much attention in recent years, one of the world's largest fertility centres, the IVI clinic in Valencia, Spain, investigated energising eggs by following proprietary procedure. To obtain the egg precursor mitochondria, an ovarian tissue biopsy was performed in the 59 study participants, who were all aged ≤42 years and had a past record of unsuccessful *in vitro* fertilisation (IVF). Stimulated eggs were randomised to injection with the mitochondria and intracytoplasmic sperm injection (ICSI) or ICSI alone, before culturing and pretransfer embryo screening.

Not only did the results show no significant difference in live birth rate per blastocyst transfer between the mitochondria transfer plus ICSI group and the ICSI alone group (41% versus 39%, respectively), the number of chromosomally normal blastocysts in the mitochondria group was significantly less when mitochondria transfer was performed. As the first prospective trial with an intrapatient comparison to assess the impact of the technique on egg quality, these results have led the IVI clinic to stop investigating this technique in these patients. "Unfortunately, the technique was not found useful for this type of patient, so we see no value for this patient population," commented Dr Elena Labarta from IVI clinic.

From these results it is clear that the technique of energising eggs still has a way to go before it can be used to improve egg quality with certainty. For example, this study involved injection of a mitochondrial solution but did not isolate the specific egg precursor cells or extract the mitochondria as performed by other groups. More work is therefore required to successfully improve oocyte and embryo quality in difficult-to-treat patients who have failed previous fertilisation techniques.

Endometrial Scratch: Time to Reconsider?

ENDOMETRIAL scratch (ES) is a common adjuvant treatment in *in vitro* fertilisation (IVF), whereby a small scratch to the lining of the uterus is made prior to implantation; it has been suggested that the resulting inflammation is conducive to successful implantation. However, new data from a large, randomised trial including >1,300 women from 13 fertility centres in 5 countries and presented at the ESHRE Annual Meeting 2018, has shown no benefit to the technique.

"...even based on just our results, I think clinics should now reconsider offering endometrial scratch as an adjuvant treatment."

The patients were randomised evenly into two groups: ES or no adjuvant treatment. The ES procedure was performed with a Pipelle® cannula (Cooper Surgical Inc., Trumbull, Connecticut, USA) between Day 3 of the preceding cycle and Day 3 of the IVF/embryo transfer cycle. Results in both groups were remarkably similar, with clinical pregnancy rates of 31.4% and 31.2% in the ES and control group, respectively; live birth rates were 26.1% in both groups. The probabilities remained comparable after controlling for various factors, even in patients who had a history of implantation failure, for whom the ES procedure has appeared to produce a benefit in previous studies. "Results from earlier studies have suggested a benefit from endometrial scratching in IVF, especially in women with previous implantation failure. However, many of these studies had a high risk of bias in their design or conduct and did not provide strong evidence," explained Dr Sarah Lensen, University of Auckland, Auckland, New Zealand.

ES is a very common procedure and, despite its association with moderate pain and bleeding, was found to be recommended by 83% of clinicians prior to IVF in a 2016 survey by Lensen et al. "Our results contradict those of many studies previously published, and, although our trial was the largest and most robust study so far, it can be difficult for one trial to change practice," said Dr Lensen. Further studies currently being performed by different groups in the UK and the Netherlands will clarify the technique's efficacy, but the researchers suggest that their data alone should warrant clinicians to reconsider the use of ES. "[...] even based on just our results, I think clinics should now reconsider offering ES as an adjuvant treatment," concluded Dr Lensen.

Evidence on the Use of Antioxidants in Treating Infertility

SHOULD antioxidant supplements be taken by men with male factor infertility? This was the question posed by researchers of a clinical trial based in the USA, the results of which were reported in a ESHRE press release dated 2nd July 2018. Although there have been previous studies that linked antioxidants to improvements in sperm quality, most of the studies have been limited, whether due to patient heterogeneity, small numbers, nonclinical endpoints, or based on the variety of antioxidant explored. Thus, a stronger evidence base is required.

The study participants numbered 174 couples, and all the men in the study had received a diagnosis of male factor infertility. At the beginning of the trial, four sperm parameters were measured: levels of sperm concentration, sperm motility, sperm morphology, and the rate of DNA fragmentation. These parameters were also measured at 3 months. Another trial endpoint was natural conception. The men in the study were allocated to either receive a placebo or treatment with an antioxidant tablet. The tablet contained folic acid, zinc, selenium, L-carnitine, and vitamins C, D3, and E.

"The results do not support the empiric use of antioxidant therapy for male factor infertility in couples trying to conceive naturally."

The results at 3 months demonstrated no significant differences in morphology, motility, or DNA fragmentation; there was a slight overall difference in sperm concentration between the two study groups. Additionally, there was no significant difference found in the endpoint of natural conception; the pregnancy rate was 10.5% for the antioxidant group and 9.1% in the placebo group. Following continued antioxidant tablet treatment or placebo for the male partner and three cycles of clomiphene and intrauterine insemination for the female partner, the natural conception rates were still comparable. The presenter of the study at the ESHRE Annual Meeting, Prof Anne Steiner, University of North Carolina, Chapel Hill, North Carolina, USA, explained: "The results do not support the empiric use of antioxidant therapy for male factor infertility in couples trying to conceive naturally."

No Link Found Between Assisted Reproduction and Ovarian Cancer

HORMONAL stimulation during *in vitro* fertilisation (IVF) has caused concern in recent years regarding an increased risk of ovarian cancer. However, results of a study reported in a ESHRE press release dated 3rd July 2018 show no causal association between the fertility treatment and the risk of ovarian malignancy; instead, female infertility may be associated with an increased risk.

"We found that the higher risk of ovarian cancer among women having assisted reproduction was only present among those with diagnosed female infertility."

The Danish study used the cancer and reproductive health registries of Denmark to match 58,472 IVF or intracytoplasmic sperm injection-treated women with 549,210 non-treated women from the general population between 1994 and 2015. Although the results showed a slight increase in the overall risk of ovarian cancer among the women who underwent assisted reproductive techniques compared to controls (0.11% versus 0.06%, respectively), these higher rates were also present in nulliparous women and in infertile women. Since fertility treatment of women who were fertile but had male partners who were infertile was associated with a lower risk of ovarian cancer, the researchers concluded that any observed increased risk in ovarian cancer was not due to hormonal stimulation of the ovaries and, instead, was more likely related to female infertility. In addition, the team noted that the increased risk of ovarian cancer was highest in the first 2 years after assisted reproductive treatment, but after 12 years the risk was similar to that of the general population; the authors suggested this pattern was due to an influence of detection bias during treatment.

"We found that the higher risk of ovarian cancer among women having assisted reproduction was only present among those with diagnosed female infertility," concluded Prof Anja Pinborg, Fertility Department, Righospitalet, Copenhagen University Hospital, Copenhagen, Denmark. Since ovarian stimulation did not increase the risk of ovarian cancer in the general population and the absolute risk of being diagnosed with the cancer remains small, Prof Pinborg described the results as "reassuring" and advised infertile women to move forward with their assisted reproductive treatment.

Trial Data Supports Anti-Müllerian Hormone Analysis for Personalised Rekovelle® Therapy

RESULTS of the Phase III ESTHER-1 trial assessing the effect of using anti-Müllerian hormone (AMH) levels to personalise follitropin delta (Rekovelle®, Ferring Pharmaceuticals, Saint-Prex, Switzerland) dosing were outlined in a ESHRE press release dated 2nd July 2018. The study data emphasised that natural variations in AMH during and between a woman's menstrual cycles has no clinically relevant impact on ovarian response when using AMH to dose follitropin delta. This further emphasises that AMH measured on any day of a woman's menstrual cycle can be used to personalise follitropin delta dose.

Follitropin delta is a human recombinant follicle stimulating hormone, approved for ovarian stimulation in women undergoing assisted reproductive technologies. Individualised dosing of follitropin delta is based on body weight and AMH, a biomarker for ovarian reserve that can predict ovarian response. ESTHER-1 results showed, in addition to validating AMH as a biomarker for personalised follitropin delta dosage irrespective of when levels are analysed in the patient's menstrual cycle, that more women receiving individualised follitropin delta treatment achieved the target response of 8–14 eggs compared to conventional dosing with follitropin alfa.

These findings will have a great impact on assisted reproductive therapies; ovarian response to stimulation can vary considerably between women and extreme responses can have implications on efficacy and patient safety. The validation that follitropin delta dosing can be individualised using AMH level analysis and body weight will ensure that extremes in ovarian response can be avoided, guaranteeing that efficacy and safety are optimised.

Prof Klaus Dugi, Chief Medical Officer, Ferring Pharmaceuticals, summarised the importance of the study results: "I believe these data will further strengthen the growing confidence of doctors in the use of AMH to personalise dosing of fertility treatment for their patients."

"I believe these data will further strengthen the growing confidence of doctors in the use of AMH to personalise dosing of fertility treatment for their patients."

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Interviews

Hear insights from across Europe in the interviews with the *EMJ Reproductive Health* Editorial Board

Featuring: Dr Ioannis Sfontouris, Dr Monica Muratori , Dr Melihan Bechir, and Dr Antonio Simone Laganà

Dr Ioannis Sfontouris @yannisfontouris

Eugonia IVF Centre, Greece

Firstly, what inspired your interest in reproductive medicine and, more specifically, embryology?

Embryology is a fascinating part of reproductive medicine, combining several sectors of biological science, including cell biology, physiology, and endocrinology. It is also very challenging because it is fast-evolving, requires the development of high-precision technical skills, includes extensive patient contact, and certainly leads to the miracle of helping create a new life! All of the above inspired me, even during my undergraduate years, to get involved with clinical embryology, and naturally I have not looked back since!

You are a Deputy of the European Society of Human Reproduction and Embryology (ESHRE) Embryology Special Interest Group (SIG). Could you detail what your role entails?

Being involved in the activities of the largest SIG of ESHRE is a very exciting way of voluntarily giving up any last traces of your free time! At the same time though, it is a very stimulating and rewarding experience. The aims of all ESHRE SIG are mainly educational. My role as a SIG Embryology Committee member includes, but is not limited to, the organisation of precongress courses and Embryology Campuses, participating in guideline and policy development, reviewing and scoring abstracts submitted to the annual meetings, and collaborating with other SIG in promoting basic science and laboratory practice.

"Embryology is a fascinating part of reproductive medicine, combining several sectors of biological science, including cell biology, physiology, and endocrinology."

To date, the Embryology SIG has focussed on the development of education within the field using precongress courses and workshops, alongside the maintenance of high standards via the implementation guidelines. What changes within the field of embryology have you noticed since joining the ESHRE Embryology SIG?

These activities reflect the constant and continuous effort of past and present SIG embryology boards. Apart from a number of successful Embryology Campuses, the most recently published contributions in the last couple of years include the update of the guidelines on good laboratory practice and the publication of the Vienna Consensus on assisted reproductive technology laboratory performance indicators. In addition, we have an ongoing survey inviting suggestions on how to further improve and develop the ESHRE online embryology atlas, so I encourage everyone to send in their comments. All this is extremely useful educational material and should be an invaluable reference guide for embryologists worldwide.

"We are constantly faced with new challenges..."

With past successes in mind, what do you think the goals of the Embryology SIG should be for the next 5 years? How will these goals be achieved?

We are constantly faced with new challenges; the list is very long. Therefore, it is the SIG's role to provide guidance in order to promote the best possible scientific standards in our profession and ultimately in the treatment of the infertile couples who seek our care. Several novel elements have been implemented in the embryology laboratory, for example, time-lapse embryo monitoring, new generation embryo culture media, different types of incubators, and new embryo selection criteria. Some of these elements we will possibly address in the form of published guidelines or consensus in the next couple of years. Although I cannot predict with certainty what will happen 5 years from now, I am confident that artificial intelligence will have made a significant entrance in the field of *in vitro* fertilisation (IVF) by then. Even in this year's forthcoming ESHRE Annual Meeting in Barcelona, Spain, I anticipate several submitted abstracts on artificial intelligence and machine learning.

In 2016, the British Broadcasting Corporation (BBC) published a report detailing how add-on therapies offered in conjunction with IVF, used to improve the likelihood of successful pregnancy, are not supported by evidence. What do you make of the findings of the report and what effect has it had on the field, particularly with regard to medical ethics?

IVF is a fast-evolving field, both technologically and medically. As a result, many treatments and interventions have rapidly reached the IVF clinic and are offered to patients without prior sufficient testing. Unfortunately, these 'modern' therapies are aggressively promoted by companies and at the same time actively marketed by IVF clinics in a race to increase profit and attract more patients. The problem is that patients are usually asked to pay large sums of money, on top of an already expensive process, for methods that are not guaranteed to work in the first place, with some interventions even being possibly harmful in some instances. There is a big discussion going on at the moment, led especially by the Human Fertilisation and Embryology Authority (HFEA) and the Fertility Network in the UK, that aims to raise awareness and educate the public, as well as medical professionals, regarding the use of add-on therapies in IVF treatment.

What could ESHRE do to combat the use of unsupported add-ons and unproven IVF techniques?

There is no doubt that the best clinical and laboratory practice should be based on evidence. This is a strong position of the Embryology SIG. In this respect, we organised a special Embryology Campus entitled "Evidence-based practice in the IVF laboratory". This 3-day event took place in Athens on 17th–19th May 2018 and aimed to provide an update on the efficiency and safety of, as well as the biological and clinical basis for, several unproven techniques, older and novel, that we use in the IVF laboratory despite controversy or insufficient evidence.

How important do you feel ESHRE guidelines and approved procedures are for ensuring a successful pregnancy and birth?

The publication of guidelines is a cornerstone contribution of ESHRE to the global community of reproductive medicine. The development of a guideline is a long and arduous process and is performed by international experts in a particular field. ESHRE guidelines include the current available evidence on the efficacy and safety of several practices, as well as critical interpretation and clear directions for scientists and clinicians. It has been shown that these guidelines make an impact on everyday practice worldwide, help promote clinical efficiency and optimisation, and ultimately safeguard the chances of a successful pregnancy and healthy baby being born.

There is growing evidence, including recent study results from the USA, Iran, Australia, and New Zealand, that IVF and other assisted reproductive technologies have detrimental effects on the future health of the offspring. What do you make of these findings and how do you think these issues can be overcome?

"...many treatments and interventions have rapidly reached the IVF clinic and are offered to patients without prior sufficient testing."

Despite the fact that the majority of epidemiological studies so far have indicated that IVF appears to be safe for the offspring, some studies are published now and again that raise new concerns. The results of all such follow-up studies reporting health outcomes of children born as a result of IVF or intracytoplasmic sperm injection should be read with a critical approach. It is true that in some cases, IVF techniques appear to be associated with a higher incidence of birth defects or other anomalies; however, usually this increase seems to be associated with the presence of infertility or advanced parental age and not with the process of IVF itself. Ovarian stimulation, fertilisation and embryo culture outside the body, cryopreservation, and pre-implant genetic screening are all significant interventions and their long-term effects are not yet fully understood. Therefore, the long-term follow-up of children should be carried out for many years, until they reach adulthood, as this is of paramount importance to determine the long-term safety of these procedures.

As a member of ESHRE, how important do you feel large congresses are for the progression of the medical field and also for personal development as a medical professional?

Large congresses, like the ESHRE Annual Meeting, attract researchers and delegates from all over the world, and are unique events that offer the latest updates on all sectors of reproduction. The broad spectrum of themes covered during ESHRE meetings ranges from embryology and genetics to endocrinology, surgery, and ethics, among others. This has been proven to form bridges and forge collaborations between scientists and clinicians from different reproductive specialities, which is essential for the progression of our field. Also, there is no doubt that there are excellent networking and socialising opportunities at an informal setting. The ESHRE Annual Meeting in 2018, which took place in Barcelona, Spain, from the 1st–4th July, was described, as usual, as the scientific event of the year.

Finally, what three pieces of advice would you give to a young scientist or medical student looking to progress within the field of reproductive medicine?

Three pieces of advice I can offer would be to study hard, be inspired, and do not give up. Things will happen for you as long as you persist and you love what you do. That goes for all things in life, including a career in reproductive medicine.

"Large congresses, like the ESHRE Annual Meeting, attract researchers and delegates from all over the world..."

Dr Monica Muratori

University of Florence, Italy

What first inspired you to develop your career within reproductive health?

I began my career within reproductive health in the late 1990s and, frankly, it was by chance. I was working on endometrial cancer and had set up some new flow cytometric techniques to study the cell growth of cancer cells. Some years before, the head of the Unit where I was working, Prof Gianni Forti, had started the research in spermatology, and I was asked to set up flow cytometric techniques to study several sperm parameters. I quickly realised that andrology and sperm biology were relatively young disciplines and, thus, it would be exciting to carry on investigations within these fields of research.

You have a particular interest in sperm biology. What first drove you to develop this interest and what about this specialism keeps you motivated?

At the beginning of my interest in sperm biology there was a question of whether mature spermatozoa could die by apoptosis. I was astonished that the fate of the millions of spermatozoa, which are released from testis each day, was not yet known. I thought that sperm apoptosis could be a suitable mechanism to regulate the homeostasis of the number of spermatozoa in the male genital tract and, similarly, to remove the many spermatozoa deposited in the female tracts, where only one is committed to fertilise the oocyte. Today, it is still unclear whether apoptosis is the mechanism by which spermatozoa are destroyed in the male and/or female genital tract; however, we know that mature sperm cells are able to trigger cell death, leading to DNA fragmentation. Based on this first interest, I began to study sperm DNA fragmentation in years when the clinical importance of this new semen parameter was emerging. These studies led to the development of a clinical service of sperm DNA fragmentation testing within the male infertility workup in my department. This field of research offers the feeling of being able to impact the clinical management of the infertile couples. On the other hand, there are still many questions to be answered about sperm functions and the fertilisation process, and so my interest is kept active.

"...it is still unclear whether apoptosis is the mechanism by which spermatozoa are destroyed in the male and/or female genital tract..."

How important is cross-discipline communication in reproductive health? Do you work closely with clinicians or researchers from other biomedical departments?

Cross-discipline communication is crucial in reproductive health, as well as in every discipline of research. Communication with other researchers is a fantastic opportunity to cultivate new ideas and solve problems in ongoing investigations. Additionally, collaboration with clinicians helps one to focus on topics that are more likely to impact patient management and treatment. In my department, the staff of the andrology unit meet periodically and, all together, researchers, clinicians, and technicians try to face the problems occurring in the daily work of the unit. These meetings also serve to frame the research activity in the clinical scenario of the infertile couple and, vice versa, to offer the expertise of the researchers to solve or ameliorate issues of clinical activity.

One of your specialisations involves DNA damage within sperm cells. How common is this damage and are there any options for treatment?

The type of sperm DNA damage that has been characterised most often in recent decades is DNA fragmentation: i.e., the presence of single and double strand DNA breaks in the

sperm nuclei. Sperm DNA fragmentation is quite common in humans, especially in infertile men or in men experiencing conditions such as diabetes, ageing, exposure to environmental toxicants, or with certain lifestyle factors. The studies investigating the impact of sperm DNA damage on reproduction produced much controversy, especially those investigating the effects on assisted reproduction. Today, we can say that the negative influence of sperm DNA fragmentation on reproduction is well-acknowledged and we can start to think how to prevent the effect of this type of sperm damage. Indeed, the causes of sperm DNA fragmentation have been characterised and include apoptosis, defects during sperm chromatin maturation, and attack by reactive oxygen species (ROS). Of the treatments that have been proposed none have showed conclusive results, even if some of these treatments appear promising, including follicle-stimulating hormone (possibly decreasing testis apoptosis) and antioxidants. In addition, preliminary results have been reported that using testicular, instead of ejaculated, spermatozoa for intracytoplasmic sperm injection ameliorates the rate of clinical pregnancy, miscarriage, and live birth.

How is reproduction affected by sperm cells with damaged DNA?

The answer to this question remained controversial until recently. Indeed, whereas the impact of sperm DNA fragmentation on natural reproduction has long been established, the studies on the effect on reproductive outcomes in couples treated by assisted reproductive techniques (ART) have often been conflicting. Controversial conclusions are mainly due to the huge heterogeneity among the studies, including the techniques used to reveal sperm DNA fragmentation. In addition, many studies neglected to standardise the female factor of infertility, which contributes to about 50% of infertile couples.

Despite this controversy, two recent, independent meta-analyses, grouping the studies according to the technique used to detect sperm DNA fragmentation, concluded that high levels of this sperm damage decreased the probability of clinical pregnancy in couples treated by intracytoplasmic sperm injection and *in vitro* fertilisation (IVF). In addition, increased amounts of sperm DNA fragmentation negatively impact on miscarriage rate in couples who conceived naturally or after ART.

What is the most interesting case you have seen that has arisen because of DNA damage within sperm cells?

The most interesting cases are those subjects showing high levels of sperm DNA fragmentation and normal values for conventional semen parameters (i.e., sperm count, sperm morphology, and sperm motility). Today, we know that sperm DNA fragmentation is a variable, independent from semen quality, as assessed by routine semen analysis. However, in the first studies this was not so clear. The finding that there were infertile subjects with high levels of sperm DNA fragmentation despite 'regular' semen quality was a clear sign that testing this type of sperm damage could provide additional information on male fertility status with respect to that offered by routine semen analysis; the latter, indeed, is not able to unveil all the sperm characteristics that are necessary to reach the oocyte and successfully support the subsequent embryo development. Sperm DNA integrity is one of these sperm characteristics that routine semen analysis is unable to detect.

"Cross-discipline communication is crucial in reproductive health..."

What are the causes of DNA damage within sperm cells? Are there any lifestyle factors that can be changed to try and avoid this damage?

As mentioned, several conditions induce sperm DNA fragmentation: ageing, diabetes, lifestyle factors, varicocele, obesity, cancer (itself or following chemo or radiotherapies), and exposure to environmental toxicants. It is believed that all these conditions induce sperm DNA fragmentation by increasing apoptotic rate in the testis and/or oxidative stress in the male reproductive tissues. Indeed, at the cellular level, the main mechanisms that have been hypothesised are testis apoptosis

and attack by ROS. Apoptosis in testis can be triggered by several stimuli, including the failure of chromatin maturation during spermiogenesis. In addition, the finding that the amount of sperm damage is lower in testicular than in ejaculated spermatozoa suggests that a fraction of DNA damage can be produced after spermiation, during the transit through the male genital tract, where an oxidative environment can occur. Indeed, it is well known that ROS exposure induces DNA breakage and spermatozoa are particularly vulnerable to the attack of these aggressive compounds.

"...one of the most exciting current fields in sperm biology is the sperm epigenome..."

As mentioned, several lifestyle factors are believed to increase the levels of sperm DNA fragmentation. They include smoking, alcohol consumption, intense mobile phone usage, physical inactivity, and obesity. Consequently, men with problems of infertility are usually counselled by clinicians to cease or change these habits. Another important source of sperm DNA fragmentation is the environmental exposure to toxicants, particularly relevant in cases of professional exposure or of living in highly polluted living areas. This is a problem of utmost importance considering the increasing contamination of water, soil, air, food, beverages, and the household all contributing to the exposure of humans to many toxic compounds.

How do you detect DNA damage in sperm cells? When should a patient be tested to check for this damage?

The techniques for detecting sperm DNA fragmentation are a very hotly debated issue. Indeed, there are several available techniques that show important differences in many aspects, including sensitivity, specificity, way to express the results, and the type of DNA damage that is revealed. Given that, it is not surprising that the clinical correlations of sperm DNA fragmentation also vary with the technique used to reveal the DNA damage, as is emerging in recent studies. I believed that the heterogeneity among these techniques greatly contributed to the confusion and conflicting data on the relationship between sperm DNA fragmentation and reproduction.

Testing sperm DNA fragmentation would be of value in several clinical scenarios, including men affected by varicocele, male partners in couples with unexplained infertility, with recurrent miscarriage, or with recurrent failure of ART. In addition, all those subjects who are exposed to the above-mentioned conditions that increase the level of this type of sperm DNA damage could benefit from sperm DNA fragmentation evaluation as well.

How would you like to see the field of sperm biology develop over the next 5 years?

I think that one of the most exciting current fields in sperm biology is the sperm epigenome and the studies on this topic will deliver huge knowledge for general and reproductive health. Epigenetics can explain how the environment interacts with genes and, when such interactions occur in germ cells, how the effects can be transmitted to future generations. In the last decades, humans have experienced a severe environmental change with an increasing exposure to many contaminants. Many cases of idiopathic infertility, currently accounting for about 40% of male infertility, might be explained by the environmental effects on sperm epigenome. In addition, as male fertility potential reflects the general human health due to the high sensitivity of germ cells to the environmental factors, the hope is that results from these studies will prompt a deeper awareness of this global problem and more incisive actions to try to stop it.

Is there any research that you are working on at the moment that particularly excites you? How will it influence the future of reproductive medicine?

Recently, I started to study several markers in the sperm from patients with cancer affecting men of reproductive age; these patients undergo semen cryopreservation to preserve their fertility before treatment with cytotoxic therapies. Indeed, such therapies are known to produce different degrees of infertility, depending on

the type of cancer and the type and duration of therapy. Cryopreservation coupled with ART gives a future chance of parenthood for these men. However, cryopreservation is noxious and it appears that the number of pregnancies achieved with cryopreserved sperm from cancer patients is lower than that of healthy men. The hope is to find early semen signs of reproductive age cancer that would represent noninvasive markers for screening and promptly identifying affected men, to ameliorate the prognosis of the disease and the chance of future fatherhood.

Dr Melihan Bechir @LinkedIn

Regina Maria Private Healthcare Network, Romania

You are a senior embryologist and have many years of experience with infertility and assisted reproductive treatments, as well as ultrasound and gynaecological surgery. What first led you to pursue this career?

For years, I have examined countless pregnant women and helped to birth a lot of children, which is always an emotional experience. This is made into an even more intense experience if the parent has gynaecological pathology. As time passed, I began to ask myself existential questions and, for me, this was the moment when the passion for embryology was born. I would not have thought I would leave the surgical scalpel in favour of the laboratory pipette.

You are a founding member of the Romanian Society of Reproductive Medicine. How has the society grown and developed since its formation, and how has your role within the society changed during your time there?

Indeed, I am one of the founding members of the Romanian Society of Reproductive Medicine, but, at the same time, I am also the vice president of the Romanian Embryologists' Association. I can discuss the activities of the Romanian Embryologists' Association in more detail because my involvement was different.

Every year I participate in the organisation of the association's symposium and I have also been a speaker at several editions. The organisation advocates for government reimbursement of infertility treatments within national health programmes. We also participate with expertise in accreditation commissions of assisted reproductive centres, and I take part in organising a national examination for accreditation in clinical embryology. In the future, we want to develop guidelines on best practices for Romanian *in vitro* fertilisation (IVF) laboratories.

"It is very true that obtaining information can be done in many ways and on different paths, but socialisation in person is important and impacts the perception of information."

How important do you feel congress attendance is for personal development and the progression of the reproductive field?

It is very true that obtaining information can be done in many ways and on different paths, but socialisation in person is important and impacts the perception of information. During these events within the field of reproductive medicine, the importance of communication and sharing information is realised in real terms. After such events, I always ask myself what information I obtained that can improve my work for the benefit of patients.

A research group from the University of Edinburgh, Edinburgh, UK, have recently announced that they were able to nurture oocytes to full maturity in the laboratory. How important is this advancement and what impact could it have on current infertility treatments?

For patients who have undergone treatment for neoplasia, in which ovarian tissue cannot be transplanted, the development of culture systems for *in vitro* growth combined with *in vitro* maturation could be a source of gametes and a solution for infertility. It is very true that it is necessary to define, technically speaking, each link of the process. In clinical practice, we look to further develop methods and techniques to provide real solutions to patients. We are aware that validation is needed in the field of research, initially, and then in the clinical field.

The work of the Dumoulin laboratory group based at Maastricht University, Maastricht, Netherlands, suggests that the type of growth media used during infertility treatment can significantly affect the birthweight of a child. What considerations need to be made when selecting an appropriate growth medium, and what is your opinion on this research?

Dr John Dumoulin and his team conducted a randomised and controlled trial comparing the effects on embryos of the different culture media used in IVF clinics, and I think it is pertinent to ask: if birth weight of newborns differs depending on the culture medium used, what other changes they can bring?

The embryo is a vulnerable biological entity during *in vitro* manipulation. It is known that culture media contain components that can epigenetically affect the embryo, the newborn child, and possibly the adult life of the offspring. Cryopreservation can determine a pattern of gene expression different from fresh embryos and explain the possible mechanisms for alteration of fetal growth and placental functionality.

There should be a control of the composition of culture media, followed by randomised studies, and each producing company should publish the complete composition of their media based on scientific considerations, and the modifications to occur after the prelabeled studies. Perhaps, in this sense, we should temper our enthusiasm for new media appearing on the market.

"There should be a control of the composition of culture media..."

On the other hand, it is not very clear whether culture media can affect birth weight due to the limited number of studies in humans; the samples are small and the studies were mostly retrospective, and the results seem to be biased. In the laboratories, the choice of culture media is based on criteria such as ease of use, clinical results, ease with which they can be purchased, and last but not least, the price.

The decision to use a culture medium must be made in accordance with the product characteristics. The manufacturer should announce changes in the formula of culture media and, as a result of this, the laboratories must reassess their media choice. Each IVF unit should track fertilisation rate, embryonic quality, pregnancy rate, implantation rate, live birth rate (which I do not doubt is happening), but it is necessary to continue to pursue the health of babies born in the following years.

What changes and improvements do you think fertility physicians and companies need to make to improve assisted reproductive technologies?

Responsible use of assisted reproductive technologies in male infertility.

Obesity is an ever-growing pandemic and maternal obesity during pregnancy is known to contribute to the development of offspring disease. What impact does obesity have on infertility treatment and, in your experience, what effect does maternal obesity have on the health of the offspring?

The incidence of obesity and being overweight has become a worldwide epidemic, with health consequences such as hypertension, diabetes,

chronic heart disease, lipid metabolism disorders, uterine cancer, breast cancer, and colon cancer. It is well known that there is an association between obesity and infertility: the prevalence of obesity in infertile women is high.

Obesity is associated with anovulation, menstrual disorders, infertility, difficulties in assisted reproduction, spontaneous abortion, and negative results in pregnancy evolution. Obesity has an impact on fertility and fertility treatment. It is known that obese women undergoing IVF treatment require higher doses of gonadotrophins, longer stimulation duration, and may respond less or atypically to ovarian stimulation with an increased incidence of poor ovarian response or a higher number of immature oocytes. Obesity is associated with low fertility rates, poor quality embryos, and higher rates of spontaneous abortion.

Only 5–10% weight loss in these women can improve reproductive performance, but for it to be effective the weight loss must be gradual and sustained. Weight loss can be achieved by changing lifestyle, dietary restriction, and changing physical activity levels. Rapid weight loss achieved by aggressive diets can be detrimental to reproductive results during fertility treatments. At the same time, the decision to postpone fertility treatment to allow for weight loss should take into account the effect of the subsequent increase in the woman's age.

It has been demonstrated that genes contribute greatly to the process leading to the accumulation of excess fat in the body and environmental factors acting through epigenetic mechanisms cause changes in gene expression and may explain the observed rapid rise in the prevalence of obesity. These environmental factors include maternal nutrition, teratogenic factors (such as pollutants, drugs, and alcohol), modified hormonal status (found in those with maternal obesity, excessive weight, and diabetes), maternal stress, and oxidative stress (maternal hypertension or placental insufficiency).

Obesity at conception and being overweight during pregnancy and postpartum are risk factors for obesity in the offspring. Obesity in the offspring can impact performance and cognitive behaviour. Current data support a negative association between high maternal BMI and child IQ, as well as the risk of depression and anxiety. Children born to obese mothers are at a higher risk of developing coronary heart disease, diabetes, stroke, and asthma in adulthood.

What do you feel are the biggest challenges currently facing the field of fertility treatment?

In the field of assisted reproduction, there are many demanding, exciting, and controversial challenges, such as:

- Nuclear transfer: A new reproductive treatment to overcome the transmission of maternal mitochondrial genetic mutations.
- Preserving fertility for all indications, from young patients with neoplastic disease to social freezing.
- Developing gametes from stem cells, which could be a solution for many cases of infertility.
- Gene therapy: An area that will have important implications in the future.

In the field of assisted reproduction, each case is challenging and, from my point of view, the individualisation of clinical and laboratory treatment is very important.

"Caffeine should also be avoided (no more than one cup per day) because it can delay a successful pregnancy and reduce egg cell quality."

What dietary and lifestyle advice would you give a couple undergoing infertility treatment to increases their chances of successful embryo implantation?

A simple but very effective measure to improve the embryo implantation rate is to lead a healthy lifestyle. A balanced diet with the right vitamins and trace elements can improve implantation chances. This change should be made at least 4 months before trying to conceive. Unhealthy habits such as eating fatty foods, consuming alcohol and nicotine, not exercising, and being stressed can cause implantation problems and should be replaced by healthier habits. Caffeine

should also be avoided (no more than one cup per day) because it can delay a successful pregnancy and reduce egg cell quality.

Whether an embryo can implant successfully depends on a variety of factors, and there are a number of measures to facilitate this process. Ultimately, nature itself still decides whether a pregnancy occurs or not.

If you could have given yourself one piece of advice during your years of training, what would it be?

I should have been working in the field of reproduction many years before I started; it is a field in which I work with dedication and pleasure, but I can honestly say I regret nothing and if I could start it from the beginning I would do exactly the same again.

Dr Antonio Simone Laganà @ASLagana

"Filippo Del Ponte" Hospital, University of Insubria, Italy

Since your first interview with us in *EMJ Reproductive Health 2.1* in 2016, how have you seen the field of reproductive medicine progress and develop? Has there been one specific advancement that has changed the way you look at the field?

The scenario of reproductive medicine is rapidly evolving; our clinical practice is moving to an evidence-based approach, taking into account several significant insights from basic sciences and translational medicine. Among these changes, the improved knowledge of genetic and epigenetic mechanisms is of paramount importance for addressing future research aims and selecting novel therapeutic targets.

You hold a number of positions within key organisations, such as your role within the European Society of Human Reproduction and Embryology (ESHRE), alongside your clinical role. Could you give us an insight into your daily routine? How do you manage to dedicate enough time to all of your responsibilities?

Although it is not easy to manage both clinical responsibilities, research activities, and tasks resulting from my position within ESHRE, I am lucky to work in a supportive environment that allows me to manage all these responsibilities in an appropriate way.

How do you regard the advancements in minimally invasive surgery for the treatment of gynaecological disorders? How could the use of minimally invasive surgeries be further improved to beneficially impact patient outcomes?

I recently started to work at the "Filippo Del Ponte" Hospital, University of Insubria, Varese, Italy, with Prof Fabio Ghezzi. In this centre, we are pursuing the excellence of minimally invasive surgery and pushing its current limits forward.

"The recent progress in prenatal genetic screening may present new opportunities to improve our current knowledge of several diseases..."

I have always been fascinated looking at the milestone changes that were achieved by many brave and progressive colleagues in the past; we should be very grateful to the 'fathers' of minimally invasive surgery who led the development and spread of this approach around the world. We should maintain these high spirits and look forward to finding new solutions to improve the surgical efficacy and feasibility of treatment.

In my honest opinion, minimally invasive surgery in gynaecology is not just a technical

challenge, it is a state of mind. We have to keep the invasiveness to a minimum; preserve fertility, despite the radicality; and, equally importantly, leave as few and as small scars as possible.

How important do you feel the advancements in robot-assisted surgeries are for the improvement of surgical procedures and patient outcomes?

Although I acknowledge the importance of robot-assisted surgery, to date almost all the gynaecological procedures (even the oncological cases) can be safely performed by a laparoscopic approach. Considering that the available data do not allow us to define robot-assisted surgery as superior than laparoscopy in terms of surgical outcomes, safety, feasibility, and cost, we should be extremely careful about drawing a firm conclusion and/or proposing recommendations regarding these techniques.

It has been suggested that the public is currently in a period of so-called 'app fatigue'. How has this change in attitude altered professional thinking regarding fertility tracking apps?

I truly think that reproductive medicine experts should inform patients about the limitations of fertility tracking apps, especially when the woman is trying to conceive. When fertility is a priority, we should offer accurate and appropriate approaches to track ovulation and improve fertilisation rate.

Earlier this year you published the article 'Fertility preservation in women with gynaecologic cancer: the impact on quality of life and psychological wellbeing'. Could you outline the key findings from this study? Do you have any plans for further research in this area?

Gynaecological cancer and its treatments affect the sexual function and psychological well-being of patients. In this scenario, the preservation of reproductive potential is central to quality of life and requires careful management. Unfortunately, there is still little information about fertility-sparing treatments in women affected by gynaecological cancers, despite growing attention by both the media and experts in the field. Undoubtedly, patients affected by gynaecological cancers at a reproductive age must be managed in referral centres with high-skilled minimally invasive surgeons and dedicated *in vitro* fertilisation units to evaluate the possibility of preserving ovarian tissue and/or oocytes before radical surgical treatments and/or chemoradiotherapy. In addition, it is of paramount importance to offer psychological support; therefore, we strongly suggest a psychologist or psychiatrist is included in the multidisciplinary team for the management of these patients.

There has been recent progress in the genetic screening of the genome that could be utilised in the monitoring off unborn offspring health, with the potential to predict the development of future disease development. What is your opinion on this research? Do you share the ethical concerns about this procedure?

The recent progress in prenatal genetic screening may present new opportunities to improve our current knowledge of several diseases and, potentially, allow early diagnosis and individualised management. Nevertheless, we should take into account that genetic data must be considered extremely confidential and protected against the risk of dissemination, manipulation, and/or fraudulent or discriminatory use by third parties.

In 2016, you stated that you hoped the year ahead would mark a great turning point for your work. Were you able to achieve your desired results? How has your research progressed since your interview in 2016?

During these years I had the opportunity to work with a wonderful research team, in collaboration with several highly specialised referral centres. In this stimulating environment, we were able to publish several interesting papers about immunological and epigenetic changes that occur during different benign gynaecological conditions. In particular, in 2017 I was particularly pleased to publish the article 'Unus pro omnibus,

omnes pro uno: A novel, evidence-based, unifying theory for the pathogenesis of endometriosis.'

What are your scientific hopes for the next 5 years, both in terms of your own work and for the field of reproductive medicine as a whole?

I hope that I will be able to continue both my research and clinical activities, with the aim of implementing new research findings into clinical practice in order to improve current surgical and pharmacologic treatments.

Finally, if you could cure one gynaecological disorder what would it be and why?

During my residency, I decided to direct most of my efforts towards improving standards of care for women affected by endometriosis because I saw the struggle caused by acute and chronic pelvic pain, infertility, and other severe symptoms and signs. Despite the efforts of endometriosis specialists around the world, we are currently unable to propose a definitive cure for the condition; for this reason, it is my dream to find a safe, feasible, and definite cure for this devastating disease.

"Despite the efforts of endometriosis specialists around the world, we are currently unable to propose a definitive cure for the condition..."

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The Evolution of Assisted Reproductive Technologies: A Modern Approach to Ovarian Stimulation

This symposium took place on 3rd July 2018, as part of the 34th European Society of Human Reproduction and Embryology (ESHRE) Annual Meeting in Barcelona, Spain

Chairperson: Bart Fauser[1]

Speakers: Elena Labarta,[2] Michael Alper,[3] Jon Havelock[4]

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2. Reproductive Medicine, IVI Valencia, Valencia, Spain
3. Boston IVF, Boston, Massachusetts, USA
4. Pacific Centre for Reproductive Medicine, Vancouver, Canada

Disclosure: During the most recent 5-year period, Prof Fauser has received fees and/or grant support from the following organisations: Controversies in Obstetrics, Gynecology and Infertility (COGI), Dutch Heart Foundation, Dutch Medical Research Counsel (ZonMw), Euroscreen/Ogeda, Ferring Pharmaceuticals, London Women's Clinic (LWC), Merck Serono (GFI), Myovant, Netherlands Genomic Initiative (NGI), OvaScience, Pantarhei Bioscience, PregLem/Gedeon Richter/ Finox, Reproductive Biomedicine Online (RBMO), Roche, Teva/Theramex, and the World Health Organization (WHO). Dr Labarta received a grant from Finox in 2016, has provided consultancy services for Ferring Pharmaceuticals, and is part of the Ferring Pharmaceuticals LIFE programme. Dr Alper has acted as a consultant, advisor, or provided speaker services for Beckman Coulter, EMD Serono, Ferring Pharmaceuticals, and ReproSource. Dr Havelock has been an advisor or speaker for EMD Serono, Ferring Pharmaceuticals, and Merck.

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Disclaimer: As of June 2018, follitropin delta is approved in Europe, Israel, Mexico, Australia, Brazil, and Canada.

Citation: EMJ Repro Health. 2018;4[1]:42-50.

Meeting Summary

In celebration of the 40th anniversary of the first *in vitro* fertilisation (IVF) baby this year, the symposium focussed on the modern-day approach to ovarian stimulation (OS). Chairperson Prof Fauser welcomed delegates with a look at the key achievements related to OS in the context of assisted reproductive technologies (ART) over the past century. Treatments have evolved from the first crude preparations to the refined gonadotrophin products available for clinical use today.

The theme of personalisation in OS was introduced by Dr Labarta, who looked at how we can use accurate biomarker measurements to assess ovarian reserve, predict ovarian response, and,

therefore, personalise treatment accordingly. Of the biomarkers currently available, anti-Müllerian hormone (AMH) has been identified as the best tool for individualised gonadotrophin dosing. AMH can also be used to drive evidence-based decisions in the choice of gonadotrophin treatment. Dr Alper presented results from the MEGASET HR trial, which investigated highly purified human menopausal gonadotrophin (HP-hMG) in patients identified via their AMH levels as potential high responders. Dr Havelock then demonstrated how AMH, along with body weight, has allowed for the development of the first dosing algorithm for tailoring treatment with follitropin delta, which has been validated in randomised controlled trials (RCT). Finally, the symposium closed with Prof Fauser concluding that, using the biomarker AMH, it is now possible to personalise not only the dose of gonadotrophin but also the choice of gonadotrophin treatment, representing important first steps in truly individualising OS.

Evolution of Gonadotrophin Preparations for Ovarian Stimulation

Professor Bart Fauser

This year marks the 40th anniversary of the first IVF baby, and this milestone is a clear reminder of just how much ART has evolved. OS in particular has come a long way since the early 20th century: development of the first gonadotrophin preparations began in 1910 and has since advanced to the present-day profile of gonadotrophin products (Figure 1).[1-6] Today, clinicians have multiple gonadotrophin preparations in their armamentarium, including follicle-stimulating hormone (FSH), luteinising hormone, and human chorionic gonadotropin. All three gonadotrophins are structurally comparable, each containing an identical alpha subunit along with a unique beta subunit, while being subject to specific and individual post-translational modifications.[7] Glycosylation is one such modification that can result in the formation of multiple isoforms of each gonadotrophin.[8,9]

Glycosylation patterns play a key role in determining the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of gonadotrophin isoforms, impacting their receptor binding affinity, bioactivity, and clearance rate.[8-10] The origin of the gonadotrophin (human-derived or Chinese hamster ovary [CHO] cell-derived) also has a significant impact on the glycosylation pattern of gonadotrophins. Human-derived gonadotrophins (such as urinary-derived products) and recombinant FSH (rFSH) derived from a human cell line have complex glycosylation patterns with a high sialic acid content; they contain a heterogeneous mixture of 2,3-linked and 2,6-linked sialic acid residues, whereas CHO cell-derived recombinant gonadotrophins have a less complex glycosylation pattern comprising 2,3-linked residues only,[1,11] resulting in different PK and PD profiles in humans.[12] With so many different gonadotrophins and protocols available to modern clinicians, the key question remains: 'How exactly do you optimise OS treatment?'

Anti-Müllerian Hormone: The Backbone to Personalising Ovarian Stimulation

Doctor Elena Labarta

Infertility clinics across the world are faced with a heterogeneous population of women with varying characteristics, phenotypes, and genotypes, but all with the same goal: to have a healthy baby. Ovarian reserve can be measured to predict a woman's response to OS, and treatment choice can be personalised based on this to maximise pregnancy success rates while minimising risks,[2] costs, and patient burden. Considering this, it is of critical importance that the most reliable measurement of ovarian reserve is employed to accurately predict ovarian response, which will ultimately ensure that there is the highest probability of a successful pregnancy.[2] Although a number of assessments have been proposed over recent years, AMH and antral follicle count (AFC) are widely accepted as the most reliable tools available for OS personalisation.[2]

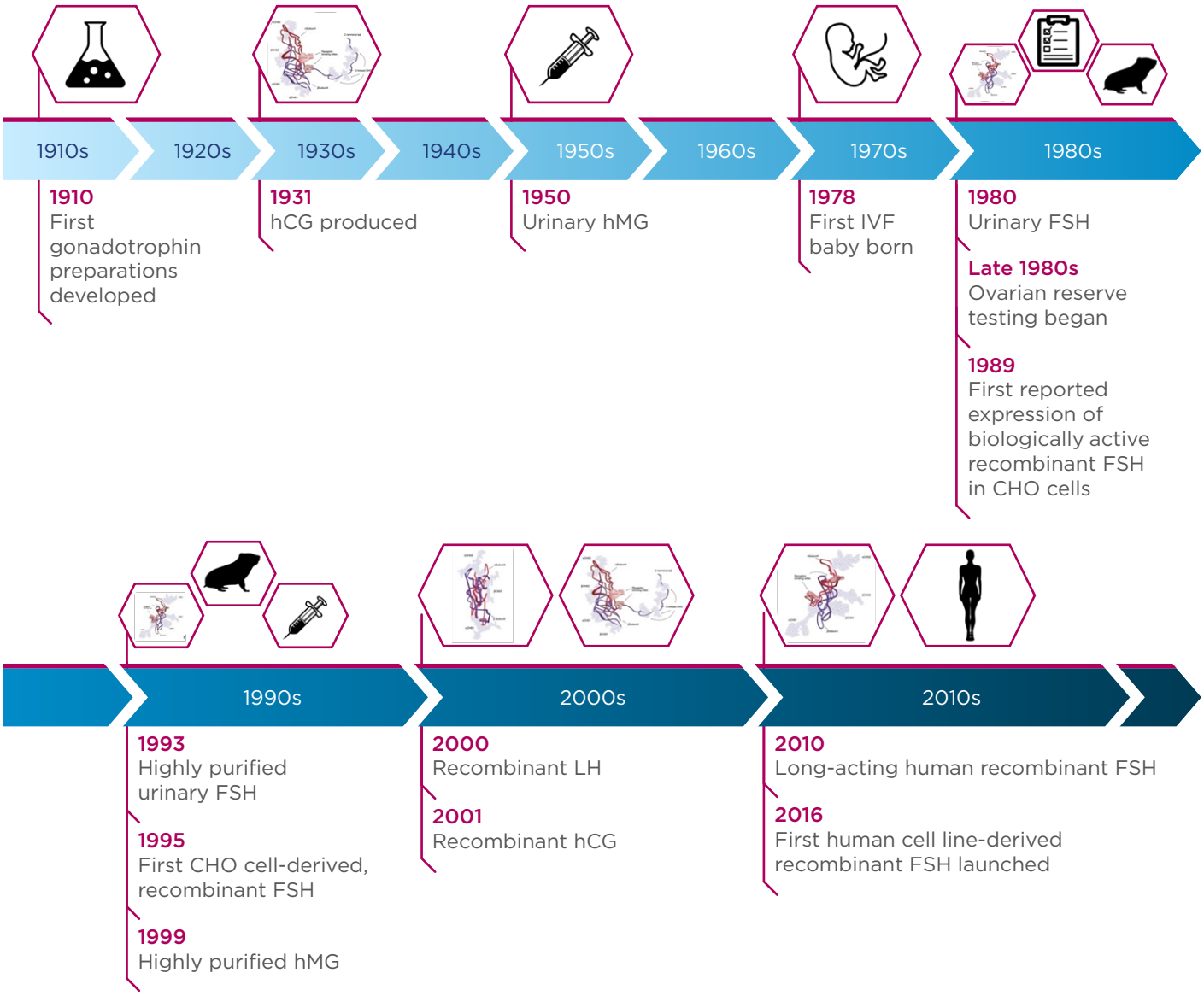

Figure 1: Timeline of gonadotrophin development from the start of the 20th century to the present day.[1-6]

CHO: Chinese hamster ovary; FSH: follicle-stimulating hormone; hCG: human chorionic gonadotropin; hMG: human menopausal gonadotrophin; IVF: *in vitro* fertilisation; LH: luteinising hormone.

FSH, hCG, and LH images adapted from Leão and Esteves.[1]

A number of analyses have been carried out to compare AMH with AFC to decide the optimal biomarker of ovarian reserve. Broer et al.[13,14] demonstrated that, in two individual patient data meta-analyses of observational trials, both AMH and AFC had similarly high performance in predicting poor and excessive ovarian response as single tests compared with age alone. When looking at AMH and AFC measurements between seven different centres, Anderson et al.[15] found that AFC showed substantial variation, whereas AMH had minimal variation when measured in a central lab with an automated assay. Moreover, secondary analyses of two large, multicentre RCT (MERiT and MEGASET)[16,17] have shown that AMH is superior to AFC for the prediction of ovarian response.[18,19] MERiT and MEGASET compared rFSH and HP-hMG in gonadotrophin-releasing hormone (GnRH) agonist and antagonist cycles, respectively;[16,17] post-hoc analyses of both trials showed that, compared with AFC, AMH correlated strongly with the number of oocytes retrieved in the majority of the centres included in these studies.[19] In addition, the MEGASET trial showed that AMH had a higher

capability for prediction of both poor and high response to OS and better performance than AFC, FSH, or inhibin B.[18] Collectively, these results indicate that AMH is less variable and has a greater correlation with ovarian response than AFC,[15,18,19] and it can be considered the single biomarker of choice for prediction of ovarian response.

Although the latter studies indicate that AMH correlates well with oocyte yield, some concerns have been raised regarding the variability of AMH levels measured as a direct result of the assay method and the stability of samples.[20] Many different assays have been used previously to measure AMH levels; however, there has been a move from manual assays (ELISA) to automated assays (Elecsys® [Hoffmann-La Roche, Basel, Switzerland], Access [Beckman Coulter, Brea, California, USA], and VIDAS® [bioMérieux, Marcy l'Etoile, France]) in recent years.[21-23] It should be noted that a lack of standardisation has been observed between the automated assays, with Access being found to systematically give higher values by an average of 10% compared with Elecsys.[24] Despite this, the automated assays have provided a solution to the issue of analytical variability and more accurate AMH measurements can now be generated in a reduced amount of time.[25] Automated assays also have a number of key advantages over manual assays; for instance, it is well known that with previous manual assays AMH was not stable under some storage or assay conditions. In contrast, Elecsys has proven to have no issues with sample instability for both sample collection type and storage conditions.[21,22]

AMH variability within individuals is another concern raised by clinicians.[20] Variability is anticipated due to biological characteristics, reproductive factors, and environmental or lifestyle factors, and clinicians should take these into consideration when assessing a patient's ovarian reserve test results.[2] AMH levels are also known to fluctuate throughout the menstrual cycle; however, fewer fluctuations have been observed in larger trials as compared with smaller studies, and the variation is not considered large enough to be clinically relevant.[25,26] It should also be noted that inter and intraindividual biological variability exists in other frequently used biomarkers, such as bilirubin, ferritin, urea, and high-density lipoprotein cholesterol,[25,27-29] and the intraindividual variability seen with AMH[26] is less than with these other biomarkers.

As well as predicting responses to OS, AMH and ovarian reserve tests can be used to personalise OS treatment;[2] patient-tailored FSH dosing using AMH as a biomarker has been demonstrated with follitropin delta.[30] To achieve this, it was necessary to first establish a dose–response relationship between exogenous FSH and ovarian response.[31] A recent Phase II trial[31] demonstrated a linear dose–response relationship with oocytes retrieved. Moreover, dose–response modelling indicated that AMH levels influence the predicted number of oocytes retrieved for various doses of follitropin delta (Figure 2).[31] The model further demonstrated that AMH levels and body weight alone were sufficient biomarkers to personalise the follitropin delta dose,[31] and this was then validated in a RCT.[30] In summary, AMH is the key biomarker for predicting ovarian response to stimulation and, with this strategy, individualisation of ART stimulation protocols (including choice of regimens and dose adjustments) is now possible.

New Insights into Highly Purified Human Menopausal Gonadotrophin: MEGASET HR

Doctor Michael Alper

Different and individualised criteria inform personalisation of OS for each patient; based on each patient's unique profile, treatment can be tailored by selecting the most appropriate gonadotrophin and deciding on the best administration dose. There is an increased emphasis in current clinical practice on selecting personalised treatment paradigms that are evidence-based and hence data-driven. Accordingly, the choice of gonadotrophin for each patient should also be evidence-based.

Patients with a high ovarian response (defined as patients who produce >15 oocytes in response to OS) experience unique problems due to the excessive production of oocytes and high oestrogen levels, which lead to an increased risk of ovarian hyperstimulation syndrome (OHSS), cycle cancellations, and a subsequent delay in time to pregnancy.[32]

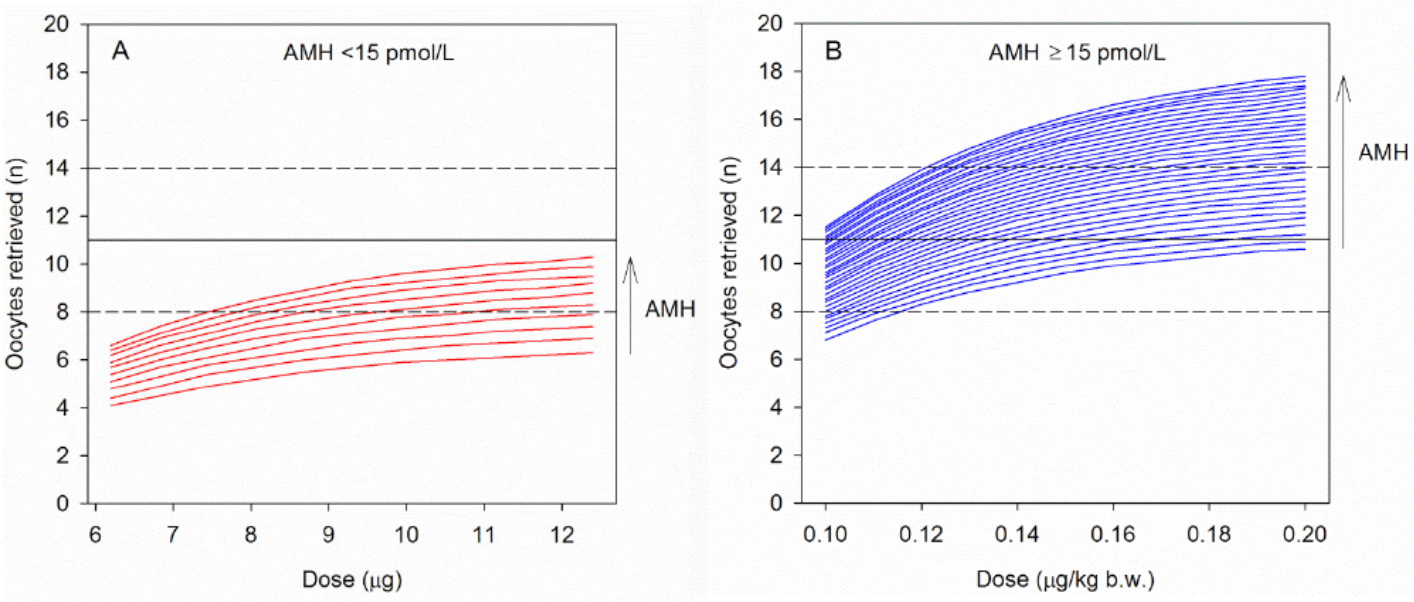

Figure 2: Dose–response model that shows the predicted number of oocytes received with different doses of follitropin delta and an anti-Müllerian hormone measurement of A) <15 pmol/L or B) ≥15 pmol/L.

The black horizontal dotted lines represent the target range of 8–14 oocytes retrieved.

AMH: anti-Müllerian hormone; b.w.: body weight.

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Previous studies (EISG,[33] MERiT,[15] and MEGASET[17]) investigated HP-hMG versus CHO cell-derived rFSH treatments in IVF and intracytoplasmic sperm injection (ICSI), and further analysis of the data provided evidence to generate the hypothesis that HP-hMG may be efficacious, with an advantageous safety profile in high responders. Advancing this, Arce et al.[34] carried out a retrospective analysis of the data collected in MERiT and MEGASET, investigating ovarian response and clinical outcome in potential high responders treated with either HP-hMG or rFSH. Results indicated that, compared with rFSH, HP-hMG was associated with a lower mean number of oocytes but a significantly lower incidence of high response (defined as >15 oocytes) and increased live birth rate per embryo transfer. The authors concluded that the specific gonadotrophin chosen for treatment has a direct effect on high response rate and, therefore, may influence clinical outcomes in high responders.[34] Many fertility experts recommend the use of GnRH antagonist protocols in high responders and those at risk of OHSS; however, there are still limited data to support which gonadotrophin should be used.[35-37]

The MEGASET HR trial[38] was set up to investigate specific gonadotrophin regimens in high responders. The study objective was to demonstrate non-inferiority of HP-hMG (Menopur® [Ferring Pharmaceuticals, West Drayton, UK]) versus rFSH (Gonal-f® [Merck Serono SpA, Modugno, Italy]) with respect to ongoing pregnancy rate in women undergoing OS following GnRH treatment. The study was a randomised, assessor-blind, non-inferiority clinical trial carried out at infertility centres across the USA. Patients predicted to be high responders (defined based on serum AMH levels) were enrolled to undergo IVF or ICSI treatment using a GnRH antagonist protocol with a fresh, single blastocyst transfer.[38] The methods and results of the study will be reported in full at a later date and are consequently not included in this symposium review.

Follitropin Delta: Ovarian Stimulation with Efficacy and Safety at its Core

Doctor Jon Havelock

Ovarian response to stimulation is variable and unexpected extreme responses have both efficacy and safety implications.[39] To minimise these risks, there is a need to predict ovarian response prior to OS.[39] The success and safety of ART depends on a balance of obtaining enough oocytes for a sufficient number of embryos to transfer while avoiding too many oocytes in order to reduce the risk of OHSS.[28] Several attempts have been made over recent years to predict ovarian response and tailor the starting dose of FSH using various biomarkers;[40-45] however, many studies have used surrogate primary endpoints for ART outcomes and trial subject inclusion criteria have not been sufficiently robust to generalise results obtained to a broader patient population. To succeed, there is a need for a data-driven model validated in a large, prospective RCT.

Follitropin delta is a unique human rFSH that differs from the existing available FSH preparations. Although it has an identical amino acid sequence to urinary and CHO cell-derived FSH, follitropin delta is the first human cell line (PER.C6® [Crucell Holland BV, Leiden, Netherlands])-derived FSH with a complex, individual glycosylation pattern that closely resembles that of natural human FSH.[46] The complex glycosylation of human-derived rFSH demonstrates a clearance rate and receptor binding profile that differs from other forms of rFSH.[8-10] Investigational studies that looked at the PK and PD profile of follitropin delta have confirmed that, in comparison with the CHO cell-derived rFSH follitropin alpha, an equal international unit (IU) dose of follitropin delta (as determined by the Steelman–Pohley assay in rats) is not equally bioactive in humans.[12] In fact, an equal IU dose of follitropin delta has a different PK and PD profile to that of follitropin alpha, resulting in a higher mean serum FSH concentration, higher oestradiol levels, and a higher median number of follicles than follitropin alpha.[12] As a result, the Steelman–Pohley assay is not appropriate for measuring follitropin delta activity in humans and, therefore, follitropin delta is dosed in micrograms.[12]

Data from a Phase I trial[47] were modelled and this revealed that the number of oocytes retrieved (when administering a constant follitropin delta dose) decreased with increasing body weight. Therefore, for the purpose of developing a validated dosing algorithm, it was most appropriate to calculate the dose of follitropin delta using body weight.[47] Furthermore, the dose–response model evaluated multiple ovarian biomarkers and demonstrated that only AMH and body weight were necessary to maximally predict the ovarian response following follitropin delta treatment.[31] Subsequently, Phase II studies were conducted to determine appropriate dosing for patients with either low AMH (<15 pmol/L) or high AMH (≥15 pmol/L), which led to the development of the follitropin delta dosing algoirithm.[31] The rationale for the development of the algorithm was to affect the predefined optimal OS to maximise pregnancy rates, while minimising the risk of OHSS or extremes of ovarian response.[30]

The ESTHER[30,48] programme, which consisted of two Phase III trials, has been carried out to support the efficacy and safety profile of follitropin delta and to prospectively validate the dosing algorithm for OS.[30] ESTHER-1 was the first study and was a randomised, multicentre, assessor-blinded, controlled, non-inferiority trial comparing the treatment strategy of individualised follitropin delta dosing with that of conventional follitropin alpha dosing for IVF/ICSI. The study used a GnRH antagonist protocol with a single blastocyst transfer, and the key inclusion criteria were women aged between 18 and 40 years with a BMI of 17.5–32.0 kg/m^2 and regular menstrual cycles of 24–35 days. The women had to be undertaking their first ART cycle and diagnosed with either tubal infertility, unexplained infertility, or endometriosis Stage I/II, or had to have partners diagnosed with male factor infertility. There were no AMH level restrictions but early follicular phase serum levels of FSH were required to be ≤15 IU/L. Ovulatory patients with polycystic ovaries were also included in the study. The coprimary endpoints of the study were ongoing pregnancy rate (10–11 weeks after transfer) and ongoing implantation rate

(a predefined non-inferiority margin of -8.0%), while the secondary endpoints included distribution of ovarian response, proportion of patients with extreme responses (hypo and hyper-responses), live birth rate, early and late OHSS, and early OHSS and/or preventive interventions.[30] ESTHER-2,[49] a continuation of ESTHER-1, was a safety immunogenicity study, allowing for up to two further OS cycles in women who did not achieve an ongoing pregnancy in ESTHER-1. In terms of the dosing of follitropin delta, this was calculated using an algorithm: in women with AMH ≥15 pmol/L, the daily dose was calculated according to the actual AMH value and body weight, while in women with AMH <15 pmol/L, a fixed daily dose of 12 µg was administered irrespective of body weight. The dosing algorithm sets the maximum daily dose at 12 µg in the first treatment cycle.[30] Once calculated according to AMH levels and body weight, the daily dose of follitropin delta was fixed throughout stimulation and was only adjusted in subsequent cycles of OS according to the response seen in the previous treatment cycle.[50]

The main efficacy results of ESTHER-1 were presented during the symposium (Figure 3): the study met its coprimary endpoints of non-inferiority, with similar data shown between follitropin delta and follitropin alpha for both ongoing pregnancy and ongoing implantation.[30] There were also similar results for the secondary endpoints of live birth rate and oocyte yield between the two gonadotrophins, though the number of oocytes obtained was more homogeneously distributed in relation to AMH levels in the follitropin delta group. In terms of OHSS and OHSS preventive interventions, with increasing levels of AMH, the risk of OHSS and/or requiring preventive intervention increased differently in the two treatment arms. These results provided additional evidence to support the use of follitropin delta with the individualised dosing algorithm.[30] Cumulative OHSS data and long-term neonatal outcomes data from ESTHER-1 and ESTHER-2 were also presented; however, these will be published in full at a later date and are consequently not included in this review. Overall, the data from the ESTHER programme demonstrate a favourable benefit–risk profile with follitropin delta treatment, especially in women with high AMH.[51]

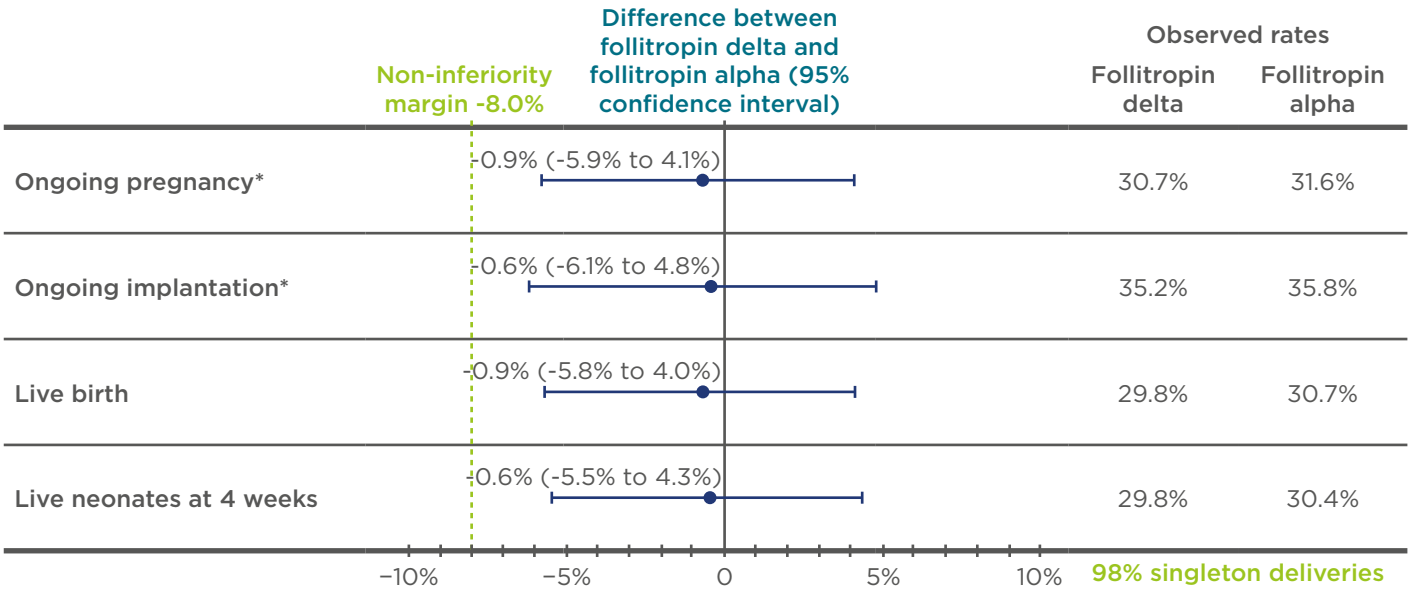

Figure 3: The outcomes of the pregnancy endpoints (ongoing pregnancy, ongoing implantation, live birth, and live neonates at 4 weeks) of ESTHER-1.

*Trial powered to at least 80% to establish non-inferiority; non-inferority limit prespecificed at -8.0% for both coprimary endpoints.

Adapted from Andersen et al.[30]

As a result of the ESTHER-1 trial, follitropin delta and its dosing algorithm have now been validated in a RCT and, to date, this is the only gonadotrophin with an approved ovarian reserve biomarker-based algorithm for dosing.[30]

It has been demonstrated that not all rFSH are the same and follitropin delta is different due to its unique PK and PD profiles. The ESTHER programme has successfully validated the safety and efficacy profile of follitropin delta. When used in conjunction with the individualised dosing algorithm based on AMH and body weight to establish a predictable ovarian response and reduce the risk of OHSS, follitropin delta provides the same pregnancy outcomes but with an improved safety profile.

Conclusion

Professor Bart Fauser

The symposium concluded with a review by the chairperson, Prof Bart Fauser, of the key points discussed. AMH is the biomarker of choice for predicting ovarian response for individualising the dose of gonadotrophins. The AMH level can also influence the choice of gonadotrophin in different patient types; this has been investigated in the MEGASET HR trial with the established gonadotrophin HP-hMG. The value of AMH in predicting OS and enabling personalised treatment can be seen in its use in the follitropin delta dosing algorithm, which uses a data-driven algorithm based on AMH and body weight and has resulted in a favourable benefit–risk profile, especially in women with high AMH levels.

As personalised treatment approaches are becoming the norm in medicine, including in infertility treatment, Ferring continues its scientific commitment to innovation in ART to support clinicians treating patients with infertility.

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Dydrogesterone: Shining New Light on Life

This symposium took place on 4th July 2018, as part of the 34th European Society of Human Reproduction and Embryology (ESHRE) Annual Meeting in Barcelona, Spain

Chairpeople: Herman Tournaye,[1] Georg Griesinger[2]

Speakers: Howard Carp,[3] Christophe Blockeel,[4] Petra Arck[5]

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Disclaimer: Dydrogesterone is not approved in all countries. Readers should check with their national regulatory body.

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Meeting Summary

This symposium took place during the 2018 Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE). It centred on the role of progestogens in the treatment of recurrent pregnancy loss (RPL) and in luteal support during assisted reproductive technology (ART), with consideration also given to the relevance of maternal adaptation in human pregnancy being under the control of progesterone and progestins. Focussing on the potential role of dydrogesterone (DYD) in the treatment of RPL, the speakers discussed the role of progestogens and how they might fit into the ESHRE guidelines for recurrent miscarriage, as the effect of this treatment approach continues to be debated. In particular, the presenters discussed whether DYD could address the current issues associated with this class of drugs; they presented evidence from the recent LOTUS I study comparing DYD with micronised vaginal progesterone (MVP) and whether the effects may be linked to supporting maternal immune adaptation for successful blastocyst implantation and the progression of pregnancy, the latter being assessed by the amount of CD4+ T regulatory cells in peripheral blood and the levels of local immune cell subsets and immunosuppressive molecules

evaluated in endometrial biopsies. There remains a need for further trials to evaluate the benefits of administering progestogens from the luteal phase of pregnancy.

Do Progestogens Fit in the European Society of Human Reproduction and Embryology Guidelines for Treatment of Recurrent Miscarriage?

Professor Howard Carp

In the current ESHRE treatment guidelines,[1] RPL is considered a homogeneous, single entity and is not classified according to prognosis or the number of losses. There is no personalised approach to classification or treatment.[1] However, opinion remains divided. RPL is defined as the loss of at least two pregnancies; furthermore up to 50% of RPL cases do not have a clearly defined aetiology.[2] Additionally, no information is provided in the guidelines about RPL patients who are resistant to treatment.[1] There is insufficient evidence to date on the benefits of progesterone, human chorionic gonadotropin (hCG), or metformin as pharmacological interventions. Similarly, there is little evidence to support the clinical benefits of hysteroscopic myomectomy, adhesiolysis, polypectomy, and intramural myomectomy. In cases of hereditary thrombophilia, antithrombotics should not be used unless indicated for venous thromboembolism. Because of insufficient benefit–risk evidence, sperm selection, lymphocyte immunisation therapy, intralipid granulocyte colony-stimulating factor or intravenous Ig, steroids, anticoagulants (heparin or low-dose aspirin), and endometrial scratching are not recommended.

With regard to progestogen treatment, there are several questions: 'Why should it work?', 'Why does it often fail?', and 'Does it actually work in clinical practice?' The basis for their predicted success is that progestogens have both endocrine and immunomodulatory functions. Endocrine effects include endometrial decidualisation, improved implantation, inhibition of arachidonic acid release leading to reduced prostaglandin synthesis, reduced cervical stromal degradation, altered barrier function to cervical ascending inflammation or infection, reduced gap junction formation, and decreased expression of oxytocin receptors.[3-5] The evidence for the role of progesterone deficiency in miscarriage is strong. A very early study by Csapo et al.[6] in 1973 showed that luteectomy before 7 weeks causes spontaneous abortion. Mifepristone blocks the progesterone receptor, causing fetal death and placental separation. Furthermore, a defective corpus luteum may produce levels of progesterone that are too low to support endometrial ripening, implantation, or placentation.

There are two main reasons why progesterone treatment may be unsuccessful. First, structural malformations in the embryo can be a confounding factor. Around 70% of miscarriages show blighted ova, and it is not possible to tell whether the rudimentary embryo may have been structurally abnormal. A study by Philipp et al.[7] using embryoscopy found developmental defects in 200 of 233 (85%) missed abortions, including anencephaly, encephalocele, spina bifida, syndactyly, pseudosyndactyly, polydactyly, and cleft hand and cleft lip. In this study, 56 out of 221 (25%) of karyotyped embryos had a normal karyotype. However, embryoscopy is an advanced technique that is not usually available; the more widely used technique, ultrasound, does not detect most anomalies (example shown in Figure 1). Another confounding factor is embryonic aneuploidy. Research has shown that 60% of sporadic miscarriages[8,9] and 45% of recurrent miscarriages[10] are due to chromosomal aberrations, including trisomies 16, 18, and 21; Turner syndrome (XO); and triploidy. In 2010, Rajcan-Saparovic et al.[11] showed copy-number variation in 26 miscarriages with normal karyotype when comprehensive chromosomal analysis was used. The incidence of embryonic aneuploidy increases with maternal age.[12]

Whether or not progesterone support can reduce RPL has been investigated in several studies, the majority of which showed positive results for progesterone, confirmed by a subsequent meta-analysis.[13] In the recent randomised, double-blind, placebo-controlled PROMISE trial of progesterone in women with

RPL (n=404 active treatment, n=432 placebo), there was a nonsignificant increase in live births in the active treatment group (65.8% versus 63.3%; risk ratio: 1.04; 95% confidence interval: 0.94–1.15).[14] MVP has no beneficial effect in women with unexplained RPL; there is some evidence that DYD may be effective if initiated when fetal heart action is confirmed.[1,15] However, as progesterone is important during implantation, DYD supplementation may be of benefit if it is administered from the luteal phase, rather than after a positive βhCG test. More trials are required to evaluate DYD and, specifically, its administration from the luteal phase onwards.

A recent randomised controlled trial of DYD (administered until Week 20 after viability was confirmed by ultrasound) showed a benefit in reducing the subsequent risk of miscarriage compared with placebo (risk ratio: 2.4; 95% confidence interval: 1.3–5.9),[15] which was supported by a subsequent meta-analysis that found DYD was favoured compared with standard treatment.[16] However, there is insufficient clinical evidence for the benefits of progesterone or hCG to reduce RPL.

Using an evidence-based approach, ESHRE guidelines recommend that progestogens should not be given if there is no evidence of effect.[1] As the medical community does not know all of the confounding factors during clinical trials, data should be evaluated using an intent-to-treat basis, relying on randomisation to neutralise potentially confounding effects. However, using a personalised-medicine approach, physicians can rule out confounding factors to reach an accurate diagnosis and determine which patients may respond (e.g., assessing karyotype of abortus or previous miscarriages and allowing the patient to decide future treatment based on this information). Key points to consider after treatment failure include karyotyping of abortus or embryoscopy if the patient was in the first trimester, noting that intrauterine embryoscopy is a specialised technique. If the embryo was abnormal, the treatment should be repeated (preferably based on karyotyping of the last miscarried embryo).

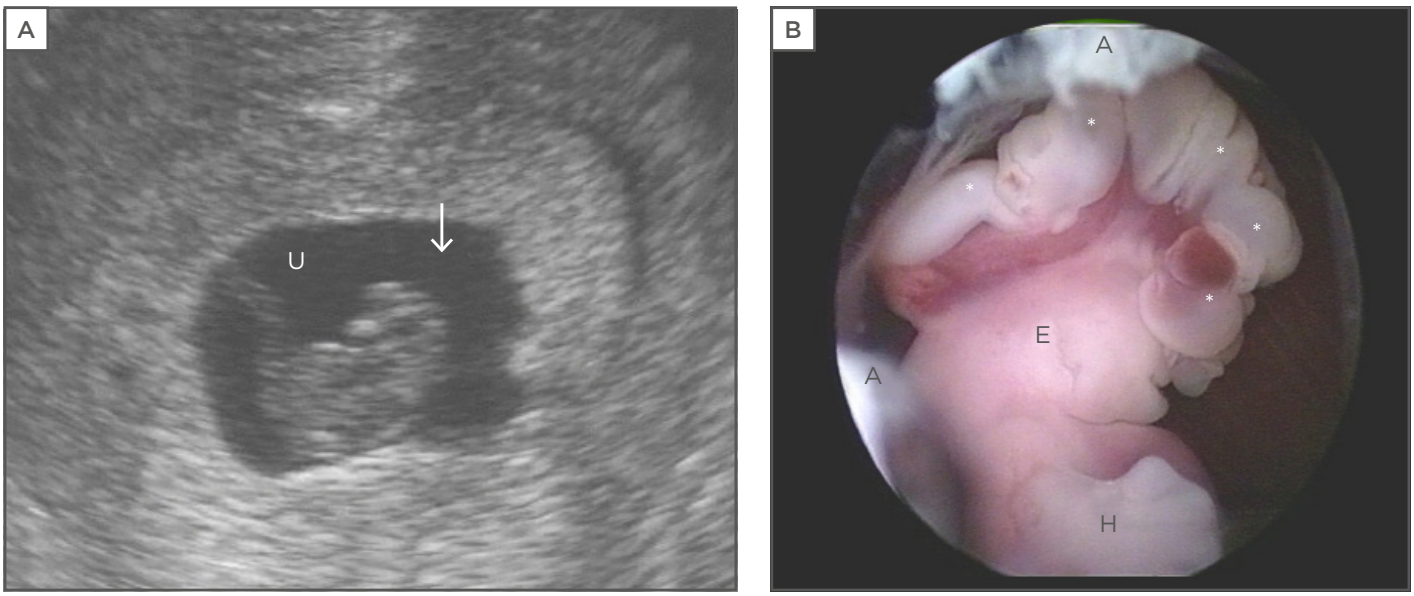

Figure 1: Structural malformations in the embryo that can lead to pregnancy loss.

Figure 1A: Endovaginal sonography prior to embryoscopy. The embryo of 17 mm, crown-rump length, showed no heartbeat. No abnormalities were identified sonographically. The arrow marks the head of the embryo. U: umbilical cord; Figure 1B: Embryoscopic lateral view of the upper portion revealed a well-preserved embryo with anencephaly. The exposed brain tissue (*) is still intact (exencephaly). The digital rays of the hand (H) are notched. Parts of the external ear (E) are clearly discernible. Remnants of the amnion are labelled (A). A normal karyotype was diagnosed cytogenetically (46,XX).

Adapted from Philipp T.[18]

Paraffin-block analysis may also be required. In the event of repeat aneuploidy, preimplantation genetic testing for aneuploidy should be employed, followed by luteal support with DYD.[17]

In conclusion, the overall findings are that progestogens may prevent miscarriage of a viable embryo, while DYD may have additional advantages over progesterone in terms of efficacy. Study results may be confounded by fetal factors, but progesterone and DYD appear to support pregnancy via both anti-inflammatory cytokine and endocrine effects. Guidelines should be used to tailor treatment to the individual patient and if the patient miscarries despite treatment, it is advisable to audit the possible causes of treatment failure.

Luteal Support in Assisted Reproductive Technology: Could Dydrogesterone Become the New Gold Standard?

Professor Christophe Blockeel

Iatrogenic luteal phase defect is caused by supraphysiological steroid levels in stimulated cycles. Following a systematic literature review[19] and a global survey,[20] with regard to luteal phase support (LS) it seems that there are currently more questions than answers: 'When to start?', 'When to stop?', 'What is the optimal duration?', 'How much support should be given?', 'What kind of support should be used?', and 'What is the optimal route of administration?'

DYD is a retroprogesterone, a stereoisomer of progesterone, with an additional double bond between carbons 6 and 7 (Figure 2).[21,22] Differences in the structure of DYD and progesterone influence the potency and potential side effect profile of these progestogens. DYD has been used globally since the 1960s for several conditions related to progesterone insufficiency.[23] It is estimated that the cumulative exposure for all indications from 1960–2017 is >113 million patients.[17] Of these, it is estimated that >20 million pregnancies were exposed to DYD *in utero*.[17] Overall, clinical and post marketing experience support the well-established and favourable benefit–risk profile of DYD in the approved indications,[23] which include progesterone deficiencies (dysmenorrhoea, endometriosis, secondary amenorrhoea, irregular cycles, dysfunctional uterine bleeding, premenstrual syndrome, threatened miscarriage, habitual miscarriage, infertility due to luteal insufficiency, and LS as part of an ART treatment) and hormone replacement therapy.[24]

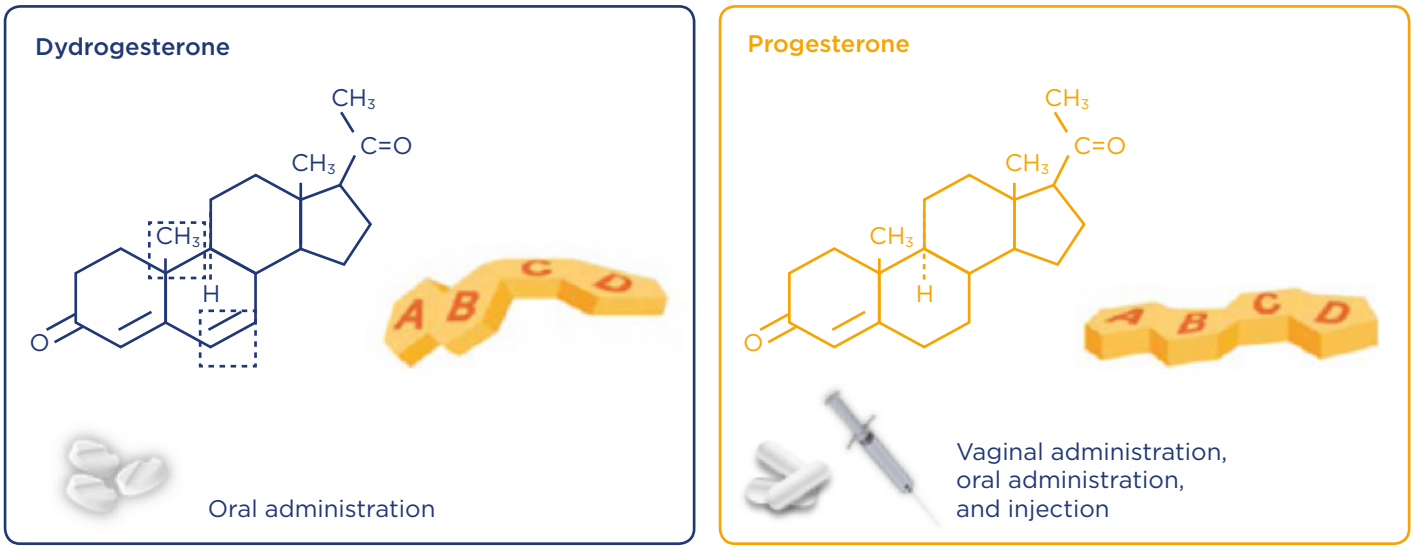

Figure 2: Differences in the structure of dydrogesterone and progesterone influence the potency and potential side effect profiles of these progestogens.[21,22]

Leading up to 2012, three prospective, randomised, controlled trials concluded that DYD was equally as effective as, or more effective than, MVP for LS in *in vitro* fertilisation (IVF).[25-27] Thus, the 30 mg daily dose of DYD for the subsequent LOTUS I study[17] was selected on recommendations of IVF specialists and based on the previous studies.

LOTUS I was a randomised, double-blind, double-dummy, multicentre, multinational study comparing the efficacy, safety, and tolerability of oral DYD 30 mg daily versus MVP capsules (600 mg daily) for LS in IVF.[17] The primary objective was the improvement of pregnancy rate (confirmed by the presence of fetal heartbeat at 12 weeks' gestation, determined by transvaginal ultrasound). Secondary objectives included a positive pregnancy test on treatment Day 15 after embryo transfer, incidence of live births, newborn assessments (sex; Appearance, Pulse, Grimace, Activity and Respiration [APGAR] score; weight; height; head circumference; abnormal findings of physical examination; and any malformations), safety, and tolerability. Demographic and baseline characteristics were similar between treatment groups. In the total treatment population (DYD n=497; MVP n=477 [full analysis set]), DYD was shown to be non-inferior to MVP regarding the presence of fetal heartbeat at 12 weeks of gestation. There was a nonsignificant, numerical difference in favour of DYD regarding live birth rates.

In conclusion, the LOTUS I trial showed that oral DYD was non-inferior to MVP for the presence of fetal heartbeat at 12 weeks of gestation, and that rates of positive pregnancy test, clinical pregnancy, live births, and newborn assessments were similar between the two treatment groups.[17] Given that oral DYD treatment had a similar safety profile to MVP in LOTUS I, with no new safety concerns identified during the study, oral DYD may replace MVP as the standard of care for LS in IVF because of the ease of oral administration.

Immunomodulation in Early Pregnancy

Professor Petra Arck

Fetal programming is an emerging concept that links environmental conditions during embryonic and fetal development with risk of diseases later in life. Mammalian pregnancy is a unique situation. The specific placental human leukocyte antigen (HLA) expression repertoire can trigger a maternal immune response, which renders the fetus susceptible to rejection. This is associated with HLA expression on trophoblast cells: a combination of negative or low expression of class Ia antigens (HLA-A, HLA-B, HLA-C), expression of class Ib antigens (HLA-E, HLA-F, HLA-G), and lack of class II antigens.[28-31] Pregnancy success results from complex adaptations, including upregulation of immunosuppressive molecules in decidual stroma cells, reduced galectin-1 expression in spontaneous abortion, the unique appearance of tolerogenic dendritic cells in the decidua of early human pregnancies, and the generation of CD4+ regulatory T cells locally and in the blood.[31-35]

The concept of Th1 (proinflammatory) and Th2 (anti-inflammatory) balance has long been thought to be important for understanding successful and failed pregnancies, but a new paradigm is emerging.[36] Balances in favour of Th1 responses can lead to epidural-associated fever, pre-eclampsia, RPL, preterm labour, and gestational diabetes.[37,38] Maternal adaptation to pregnancy is modulated by progesterone and other progestins. For example, systemic DYD administration upregulates decidual galectin-1 expression in mice, progesterone robustly increases the frequencies of CD4+ T regulatory cells in mice, progesterone and DYD support the tolerogenic phenotype of dendritic cells and trigger the release of immunosuppressive molecules in mice, and decreased progesterone levels are associated with increased fetal loss and low levels of both galectin-1 and CD4+ T regulatory cells.[39-42] Using high-parameter functional profiling by mass cytometry, combined with a single-cell signalling-based Elastic Net algorithm, it has been shown that there are dynamic changes in the peripheral immune system during the course of pregnancy.[43]

Immune adaptations are finely tuned to an 'immune clock' that regulates immune cell function to maintain pregnancy.[43] Analysis of these interrelated, chronological immune events has revealed the critical role of the IL-2-dependent STAT5ab signalling pathway in modulating T cell function during term pregnancies.[43] This has also led to an understanding of how deviations from normal immunoregulation can lead to adverse outcomes in pregnancy. Thus, the future of obstetric care to reduce RPL would involve a multidisciplinary team, including not just paediatricians, nurses, and obstetricians but also immunologists, reproductive immunologists, reproductive endocrinologists, laboratory medicine specialists, gene therapy specialists, and biostatisticians.

In summary, maternal adaptation to pregnancy is under the control of progesterone and progestins. Systemic progestogen supplementation may significantly support the maternal immune adaptation for successful blastocyst implantation and pregnancy progression. Candidates to evaluate the systemic effect of progestogens on maternal immune adaptation in human trials should include monitoring of both CD4+ T regulatory cells in peripheral blood and local immune cell subsets and immunosuppressive molecules in endometrial biopsies.

Conclusion

To conclude, the management of RPL is evolving. Current guidelines do not use a personalised approach to treatment, as RPL is classified as a single entity with no consideration of the multitude of underlying causes. There is evidence for the beneficial effects of progestogens in RPL, in particular at an early stage of pregnancy (LS), with DYD showing advantages over standard MVP, in part because of the ease of oral administration. There is also a strong immunological rationale for systemic progesterone supplementation to increase blastocyst implantation and the progression of pregnancy, demonstrated by monitoring CD4+ T regulatory cells, local immune subsets, and immunosuppressive molecules. Further studies to investigate the evolving treatment of RPL are warranted.

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Abstract Reviews

Learn more about the reproductive health studies currently underway from this hand-picked selection of ESHRE 2018 abstract summaries

Detection of Mitochondrial Reactive Oxygen Species in Live Spermatozoa of Infertile Subjects and Cancer Patients

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Cancer patients, comet assay, flow cytometry, infertile men, semen oxidative stress.

Citation: EMJ Repro Health. 2018;4[1]:58-59. Abstract Review No. AR1.

Oxidative stress occurs when the levels of reactive oxygen species (ROS) overwhelm antioxidant defences and has been strongly associated with male reproductive dysfunction. Hence, determination of ROS levels in semen is an important test for the assessment of male infertility. In this study, the percentage of live spermatozoa with oxidative stress (LOS) in native semen samples was evaluated using a flow cytometric technique, coupling the detection of sperm mitochondrial ROS with the MitoSOX™ probe (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with staining of dead cells using the LIVE/DEAD Fixable Dead Cell Stain kit (Thermo Fisher Scientific). Using this technique, LOS was evaluated in 80 infertile subjects and 16 cancer patients (testicular and haematological cancers); these patients were selected during routine semen analyses and cryopreservation of semen before cytotoxic therapy, respectively.

In infertile patients, the median LOS value was 24.80% (interquartile range [IQR]: 16.29–33.20). After grouping the patients according to the presence or absence of clinical signs of oxidative stress (inflammation and infection, smoking

habit, leukocytospermia, semen viscosity, and semen bacteria), we found that the value of LOS was 28.56% (IQR: 25.01–40.79) in subjects with clinical signs of semen oxidative stress (n=42) and 17.18% (IQR: 12.18–21.71) in subjects without clinical signs (n=38; p=0.0001). To verify whether the percentage of LOS was able to identify patients with semen oxidative stress, we constructed receiver operating characteristic curves. It was found that the LOS percentage predicted the presence of clinical signs of oxidative stress with a good accuracy (area under the curve: 0.799; confidence interval: 0.692–0.906) and that when using 22.74% as a threshold value, the true-positive proportion was 86%, whereas the false-positive proportion was 21%.

Oxidative stress is one of the main mechanisms through which sperm DNA fragmentation (sDF) occurs. To verify whether live spermatozoa with mitochondrial ROS also exhibited higher levels of sDF, first live spermatozoa with and without mitochondrial ROS were sorted using a BD FACSAria™ (Becton Dickinson Biosciences, Franklin Lakes, New Jersey, USA) cell sorter. Subsequently, the two sorted fractions were processed with a comet assay. As expected, a higher amount of sDF in the spermatozoa with mitochondrial ROS was found compared to those without (median percentage tail intensity: 37.80±9.20% versus 27.90±4.80%, respectively; p=0.06).

In the 16 cancer patients, it was found that the LOS value was 41.53% (IQR: 28.71–62.10) (testicular cancer: n=9; 44.14% [IQR: 34.26–80.32]; haematological cancer: n=7; 31.22% [IQR: 18.00–47.26]), a value much higher than that observed in infertile subjects (p=0.0001), even when considering only those presenting with clinical signs of semen oxidation (p=0.017). No difference was found between patients with the two types of cancer (p=0.174).

In conclusion, this study shows a new flow cytometric technique for evaluating oxidative stress in live spermatozoa. Contrary to previous similar methods, this technique, does not use selected spermatozoa but instead investigates native semen samples, which are more representative of the *in vivo* condition. In addition, the procedure resulted in the identification of subjects with clinical signs of semen oxidative stress with a good accuracy. With this technique, it was found that semen from cancer patients exhibited very high levels of oxidative stress, which could explain the more detrimental effects of semen cryopreservation observed in these patients.

To Flush or Not to Flush: A Retrospective Analysis of Follicle Flushing during Oocyte Retrieval in Wales

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Keywords: Eggs collection, follicular flush, *in vitro* fertilisation (IVF).

Citation: EMJ Repro Health. 2018;4[1]:59-61. Abstract Review No. AR2.

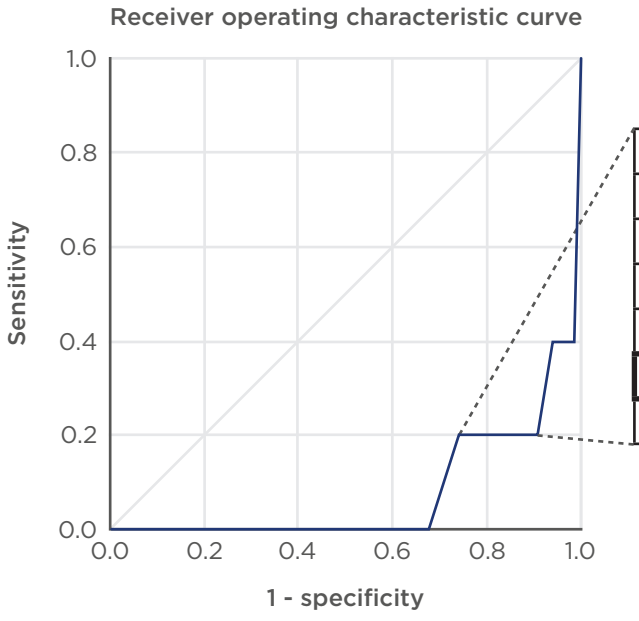
The practice of follicle flushing has been a topic of discussion since ultrasound-guided transvaginal oocyte retrieval was introduced

in 1981.[1] Theoretically, flushing would allow for the retrieval of more oocytes and consequently result in greater fertilisation and pregnancy rates. However, many studies have found no significant increase in fertilisation following the use of flushing.[2,3] The National Institute for Health and Care Excellence (NICE) guidelines[4] suggest that follicle flushing for women with three follicles prior to oocyte retrieval should not be offered, as it increases the length of the process and the pain experienced without the added benefit of greater numbers of oocytes retrieved or higher pregnancy rates.

Data from 221 women who underwent oocyte retrieval at the University Hospital of Wales, Cardiff, UK between May 2016 and April 2017 were retrospectively evaluated. In total, 162 women were included in this study; 59 had to be excluded due to inadequate information available. Thirty-nine women (24.1%) were noted to have undergone oocyte retrieval with direct aspiration only, while the remaining 123 (75.9%) had undergone oocyte retrieval with follicle flushing.

Procedures at the centre were performed using 16-gauge double lumen needles. The flushing medium used was from Origio Ltd., Reigate, UK. Chi-square tests performed with $p<0.05$ were considered statistically significant. To determine a cut-off value for the number of follicles needed to be present at the point of human chorionic gonadotropin administration, a receiver operating characteristic curve was plotted and the area under the curve was calculated. SPSS statistical software (version 24), IBM, Armonk, New York, USA, was used.

The total number of oocytes (13.6±6.5 versus 7.7±5.2), the number of mature oocytes (11.7±6.4 versus 6.3±4.6), the number of fertilised oocytes (7.5±5.3 versus 4.4±3.6), the duration of procedure (21.8±7.3 minutes versus 20.2±5.6 minutes), as well as maturation (84.7% versus 83.7%) and clinical pregnancy rates (30.8% versus 22.8%) were found to be higher in the direct aspiration only group compared to the flushing group. The chi-square test indicated that all of the above, except the duration of procedure and clinical pregnancy rate, were statistically significant.



Co-ordinates of the curve

Positive if ≥	Sensitivity	1 - specificity
4.50	0.400	0.936
5.50	0.200	0.904
6.50	0.200	0.841
7.50	0.200	0.809
8.50	0.200	0.739
9.50	0.000	0.675

Area under the curve

Area	95% confidence interval	
	Lower bound	Upper bound
0.080	0.000	0.181

Figure 1: Receiver operating characteristic curve with the suggested cut-off point of 8.50 follicles and corresponding co-ordinates highlighted. Also shown is the area under the curve.

On the contrary, the flushing group demonstrated higher fertilisation rates (57.8% versus 54.7%) when compared to the direct aspiration only group. However, these rates were not statistically significant. Figure 1 shows the area under the curve was measured to be 0.080 with 95% confidence interval (lower bound: 0.000; upper bound: 0.181). This area, being <0.5, implies that the cut-off value of approximately nine follicles at the point of human chorionic gonadotropin administration, below which follicle flushing would be recommended to obtain at least three mature oocytes, may not be accurate.

The suggested cut-off value of roughly nine follicles at the point of human chorionic gonadotropin administration, below which follicle flushing would be recommended to obtain at least three mature oocytes, derived from the receiver operating characteristic curve, may not be accurate due to a small area under the curve of 0.080 and the lower number of oocytes, mature oocytes, and fertilised oocytes in the flushing, compared to the direct aspiration group. Fertilisation and clinical pregnancy rates were not different between the two groups.

These findings, coupled with the first principles of surgery, which dictate that shorter and simpler procedures with minimal tissue handling can result in fewer complications, form the basis of our recommendation that follicle flushing should not be routinely performed during oocyte retrieval.

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Long-Term Psychological Impact of Interrupted Fertility in Cancer Patients: A Systematic Review Informing on an Improved Model of Care

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Keywords: Cancer, fertility, mood disorder, psychological distress, reproductive concerns, oncofertility, oncology.

Citation: EMJ Repro Health. 2018;4[1]:61-62. Abstract Review No. AR3.

Currently, oncofertility guidelines recommend fertility counselling at the time of cancer diagnosis to assist in fertility preservation decision-making.[1,2] A systematic review was conducted to assess the level of fertility-related psychological distress experienced by cancer patients of reproductive age (<45 years) across oncological treatment time points: diagnosis, treatment, and survivorship. This review was able to inform on a model of longitudinal care having assessed the fertility-related psychological impact that may persist into survivorship.

Results indicated both a prevalence and persistence of fertility-related psychological distress and reproductive concerns that are associated with negative emotional responses. Reproductive concerns and impacted fertility affect the sense of self and life narrative of cancer survivors, leading to a life with reduced meaning and purpose. Heightened anxiety, depression, and trauma reported at diagnosis appear to remit throughout oncological treatment, while reproductive concerns persist. However, an increased prevalence of mental health disorders was noted in cancer survivors; namely, depression in male and female survivors (22–30%), and trauma commensurate with post-traumatic stress disorder experienced by female survivors (20–72%). Findings highlight that there are risk factors for the experience of mood disorders in survivorship, including reproductive concerns, being childless, expressing an unfulfilled desire for a child, sexual dysfunction, and ovarian failure.

Discussions brought forward at the European Society of Human Reproduction and Embryology (ESHRE) 2018 Annual Meeting pertained to the clinical implications of these findings. Although there is variance in the level of distress that patients experience, the increased prevalence of clinically significant distress in survivorship highlights the need for ongoing psychological care. As such, it is recommended that all patients continue to have access to fertility counselling throughout cancer treatment and survivorship. Ongoing access to fertility information and supportive care, which form part of fertility counselling, may serve to reduce levels of psychological distress and may mitigate the likelihood of mental health disorders developing in survivorship.

Moreover, it is recommended that the provision of specialised mental health treatment be available to those patients that report significant levels of distress. Currently, models of care vary worldwide in both the availability and utilisation of fertility counselling.[3,4] At times, fertility counselling is undertaken by medical fertility specialists,[5] while, in other locations, counselling is undertaken by mental health clinicians.[6] As such, the content discussed and the training of clinicians in delivering counselling may vary widely. Results indicate the necessity of an experienced clinician being available to undertake the assessment and treatment of psychological distress and mental health disorders. As such, fertility treating centres should ensure they are able to access or refer to appropriate psychological services for those patients who report additional fertility-related psychological distress.

In addition, it is useful to consider that the experience of fertility occurs within a family system. In this sense, fertility-related distress may be experienced by family members of cancer patients, including parents and partners, or others involved in direct patient care and fertility treatment decision-making. It is advisable that when fertility-related psychological distress occurs in cancer patients or those family members involved in patient care, that fertility counselling would be beneficial to all parties.[7] As such, psychological support should be delivered to those individuals who necessitate additional care when fertility distress is identified and is not contingent on a cancer patient's specific age.

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A Comprehensive Two-Centre RNA-Seq Study Reveals Changes in Endometrial and Blood miRNome at Mid-Secretory Phase in Fertile Women and in Patients with Recurrent Implantation Failure

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Keywords: Endometrial receptivity, female infertility, microRNA, recurrent implantation failure (RIF), small RNA-seq.

Citation: EMJ Repro Health. 2018;4[1]:63-64. Abstract Review No. AR4.

The molecular changes in the endometrium that are involved in the establishment of the window of embryo implantation (WOI) are of interest for determining the reasons for recurrent implantation failure (RIF) in some *in vitro* fertilisation (IVF) patients. Genome-wide gene expression studies performed on endometrial samples, however, have several limitations: firstly, the study groups are small and the results from one research centre are poorly reproducible by another;[1] secondly, high-throughput sequencing studies on gene expression regulation by microRNA have only recently started to emerge, and also include only small sample numbers.[2,3] There has been no indication of whether molecular markers revealing optimal WOI time could also be identified from blood.

To address these concerns, the authors collected endometrial and blood samples from two independent research centres, one from Estonia (EST) and one from Spain (ESP). All study participants performed urinary ovulation tests (LH-tests). Altogether, 39 fertile volunteers (women with a history of at least one live birth) donated samples twice: 2 and 8 days after obtaining a positive LH-test (LH+2 and LH+8 timepoints, respectively). In addition, 38 RIF patients (women with a history of ≥3 unsuccessful IVF procedures involving embryo transfer) donated their samples at LH+8. All samples underwent genome-wide mRNA and small RNA deep sequencing (Illumina Inc., San Diego, California, USA). Results were compared between LH+2 versus LH+8 timepoints in the group of fertile women and between fertile versus RIF women at LH+8. MicroRNA with a statistically significant change in expression levels were further passed into downstream gene ontology analysis, during which potential microRNA target genes were sought from differentially expressed mRNA from the same samples. Only consistent results between EST and ESP centres are reported.

The authors observed that the expression of 91 microRNA changed in the endometrium of fertile women during the establishment of WOI when LH+2 and LH+8 timepoints were compared. These microRNA are involved in processes like glucocorticoid receptor, oestrogen receptor, and growth hormone receptor signalling, among

others. In addition to already known microRNA, a novel microRNA sequence was identified, the expression of which was increased 37 times in LH+8 samples compared to LH+2 samples. Bioinformatic target prediction algorithms suggest that this microRNA is involved in regulating cell cycle progression.

No differences in microRNA levels were observed when blood samples were compared between LH+2 and LH+8 samples from fertile women. A similar result has been previously demonstrated for blood plasma microRNA, confirming that endometrial cyclic changes do not reflect in systemic miRNome.[4]

When the endometrial samples from fertile women were compared to RIF patients, 21 microRNA showed significantly different expression levels. These microRNA are predicted to be regulators of the STAT3 and CDK5 signalling pathways. In addition, the level of hsa-miR-30a-5p was significantly higher in the blood samples of RIF patients. However, the molecular background of this finding still needs to be elucidated.

In conclusion, our study confirms several known microRNA, reveals novel candidates as molecular markers for WOI, and sheds light on cellular processes that are perturbed in the endometria of RIF patients. Since the reported findings are based on larger study groups and two independent population cohorts, we expect that these results can be successfully replicated by other centres. The use of these markers in the clinical setting, however, still needs to be validated.

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Young Men's Thoughts on Factors Influencing Timing of Family Formation

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Keywords: Family formation, fertility awareness, qualitative interviews, timing, young men.

Citation: EMJ Repro Health. 2018;4[1]:64-65. Abstract Review No. AR5.

Parental age has increased over the last few decades among men and women in high-income countries, including Denmark and Sweden.[1,2] Postponing family formation to the mid-30s and beyond increases the risk of age-related infertility and having fewer children than wanted.[1] Previous qualitative studies have shown that men expect to have children and take their

fertility for granted.[3] Little is known about the views of young men on the optimal timing of family formation or the factors that influence their opinions. Therefore, the aim of this study was to explore the thoughts of young men regarding parenthood and the factors influencing their views about the timing of family formation.

In this qualitative study, semi-structured interviews were conducted with 12 men from Sweden and 17 men from Denmark. Both of these countries are classified as high-income countries with similar social policies enabling people to combine paid work and parenthood. Inclusion criteria for this study were: male sex, childless, aged 20–30 years, and in the last year of education. The interviews were conducted between February and September 2017 and lasted between 30 and 90 minutes. The interviews were recorded and then transcribed and analysed through thematic content analysis.[4]

The results of this study showed that the young men valued parenthood and wanted children in the future. Factors that were identified as barriers for having children during the most fertile years were respondents associating parenthood with loss of freedom, wanting to have a secure and stable life before having children, and wanting to reflect the family formation patterns of friends and family. All respondents wanted to be in a stable relationship with the 'right' woman before contemplating fatherhood. In addition, they described a 'true order of life events' in which they expected to pursue several other life goals before having children; e.g., completing education, having financial security, and establishing a career. Most respondents did not believe that interventions to promote family formation in early adulthood would affect their preferred timing of parenthood. They had already planned what they wanted to achieve in their life, with or without children, many years ago; however, better parental leave policies and learning about risk factors for infertility in primary or secondary school were mentioned as factors that could encourage earlier family formation.

The findings suggest that young men in Scandinavia expect to achieve other life goals before being ready to have children, potentially putting them and their partners at risk of age-related infertility. The inclusion of information about the limitations of fertility in school sexual and reproductive health education may improve men's understanding of the risks of age-related infertility and promote earlier family formation.

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Follow-Up Study of Women of Reproductive Age: The Impact of Fertility Assessment and Counselling Sessions

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Keywords: Childbearing, fertility assessment and counselling, fertility, women.

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Postponing parenthood is becoming increasingly more common in high-income countries, both among women and men, and the average age of a parent at the birth of their first child has been increasing. There are knowledge gaps related to fertility issues, which include the impact of age on fertility and the success rate with fertility treatment. Earlier interventions have used standardised interventions and generally there is a lack of follow-up.[1-4] The negative consequences of delayed childbearing may be reduced by fertility assessment and counselling.

The Fertility Assessment and Counselling (FAC) clinic at Rigshospitalet, Copenhagen, Denmark opened in 2011; it is a personalised fertility awareness intervention for self-referred women and men. Women and men receive counselling regarding their fertility risk factors and ovarian reserve or semen quality.[5] We wanted to understand the impact of attending fertility assessment and counselling sessions on fertile women's decisions and subsequent choices regarding their childbearing 1 year after consultation. We have previously conducted a qualitative study exploring attitudes towards family formation in 20 women attending the FAC clinic.[6] In this study we interviewed the same sample of women 1 year after the consultation at the FAC clinic. We conducted qualitative interviews using a semi-structured interview guide. The 20 women were aged 35–40 years and were residents in the Capital Region of Copenhagen, Denmark. The interviews took place in their own homes or at the FAC clinic. We used qualitative content analysis and Lincoln and Guba's guidelines,[7] and the consolidated criteria for reporting qualitative research (COREQ)[8] were used.

We interviewed 20 different women and obtained 20 different stories. The findings highlighted the individual aspect of fertility, with every woman being a unique case. Seven women had started fertility treatment (with their partner or as a future single mother), two had left their partner, and three had delivered a baby. The overall theme was 'knowledge increased'. After the women had attended the FAC clinic, they increased their knowledge on fertility-related issues. The subthemes were 'Catalyst for change', 'Staying in limbo', and 'Peace of mind'. Some of the women saw the counselling as a catalyst for change; they made changes to their behaviour, relationship, or emotional state, and these changes were viewed positively. A few of the women felt that they were still in limbo as they were in doubt concerning childbearing. Being in limbo was experienced negatively. The women wanted concrete answers about their fertility status; they wanted an exact deadline and this was not given them. The rest of the women felt peace of mind regarding their decision-making about childbearing. The women felt that they were given time and felt less pressure to act immediately.

The FAC clinic focusses on each person and provides personalised fertility information and guidance. The knowledge the women gained served as a cue to action; it was a catalyst for change. The FAC clinic offers an individualised approach, which is needed given the unique nature of childbearing decisions.

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Female Adolescents and Young Adults with Cancer May Face a Higher Risk of Infertility Later in Life

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Female adolescents and young adults (AYA, 15–39 years) with cancer are a unique population because this age range encompasses most of the reproductive life span. Advancements in cancer treatments have contributed to significant increases in survival times; nonetheless, quality of life among survivors is also a priority. Uncertainty about future fertility has been described as a distressing factor for this population.

While some studies have reported a decrease in pregnancy rates in survivors of certain cancers, to our knowledge the risk of infertility after cancer diagnosis has not been studied at the population level in AYA.[1-4] To this end, we conducted a population-based study in the province of Ontario, Canada, to evaluate the risk of infertility in AYA survivors of selected cancers.

Using health administrative databases, we identified 15,107 female survivors of brain, breast, haematological, head and neck, thyroid, melanoma, colorectal, or urological cancer, who were diagnosed with cancer at a mean age of 31.2 years (standard deviation [SD]: 6.3). These women were compared to 64,315 cancer-free women. Both groups were followed-up for approximately 14 years. Infertility diagnosis after 1 year of cancer was identified using physicians' billing codes (International Classification of Diseases [ICD]-9 code: 628). Women with infertility previous to cancer diagnosis were excluded. Log-binomial regression models were used adjusting for sociodemographic factors (adjusted relative risk [aRR]).

Overall, the frequency of infertility diagnosis was higher in cancer survivors compared to unexposed women (12.0% versus 9.4%; p<0.001), at a mean age of 34.5 years (SD: 5.7) in survivors and 34.9 years (SD: 5.5) in unexposed women (p<0.001). Survivors of breast (aRR: 1.38; 95% confidence interval [CI]: 1.23–1.55), haematological (aRR: 1.42; 95% CI: 1.28–1.59), thyroid (aRR: 1.17; 95% CI: 1.08–1.27), and melanoma (aRR: 1.13; 95% CI: 0.99–1.30) had a higher risk of infertility diagnosis than women without cancer. These associations were stronger in nulliparous women (i.e., no

previous pregnancies) compared to women with previous children. In addition, we conducted multiple sensitivity analyses that reaffirmed our results.

Our study had limitations. Firstly, the accuracy of infertility diagnosis using ICD-9 codes in administrative datasets has not been validated. Secondly, there are nonbiologic factors that could influence the likelihood of seeking a fertility assessment that may not be captured in our study. Finally, information regarding cancer stage or treatment was not available for this analysis.

We concluded that AYA with cancer should have access to specialists in reproductive health for surveillance, and that prospective research studies should be conducted to monitor the reproductive function of this population. While the evidence is stronger for women diagnosed with breast and haematological cancer, our finding of a potential effect of thyroid cancer or melanoma needs to be further studied before any conclusion can be made. Factors other than the type of treatment could play a role on the risk of infertility, including the distress caused by cancer diagnosis, or the pathophysiology of cancer itself, including genetic factors that could be associated to both cancer diagnosis and infertility.

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A Pilot Study Investigating the Concentration of Colony-Stimulating Growth Factor in Uterine Flushing as a Prognostic Criterion of *In Vitro* Fertilisation Cycle Outcome in Patients with Recurrent Implantation Failure

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PRESENTATION SUMMARY

The concept of recurrent implantation failure (RIF) has been expanded, and the tactics to identify RIF patients have included searching for prognostic criteria of *in vitro* fertilisation (IVF) outcome, with the aim of minimising the number of unsuccessful attempts and the risk of patient drop-out. However, RIF studies are limited because most suggested methods require endometrial biopsy and subsequently

do not allow fresh embryo transfer. Therefore, there is still need for a noninvasive RIF model for prediction of IVF cycle outcome.

Granulocyte colony-stimulating factor (G-CSF) belongs to the family of haemopoietic growth factors. With regard to reproductive physiology, G-CSF has several valuable functions. In women with a normal menstrual cycle, G-CSF leads to leukocyte accumulation in the follicle and accelerates ovulation.[1] Given this fact, the administration of recombinant G-CSF was successfully implicated as a preventive tool for luteinised unruptured follicle syndrome.[2] Moreover, G-CSF is known to play a mediatory role in the process of oocyte maturation, demonstrating the direct correlation between the number of competent oocytes and the G-CSF level in follicular fluid.[3,4] Early studies demonstrated that G-CSF promoted the proliferation of trophoblast cells, thus programming appropriate functioning of the fetal–maternal interface.[5] Moreover, G-CSF seems to facilitate embryo competence. Adding G-CSF to embryo culture has benefits, including increasing development and post-transfer survival, as well as decreasing pregnancy loss.[6]

The aim of the present study was to evaluate whether G-CSF could be used as a reliable prognostic criterion of clinical pregnancy in 'fresh' cycles. An open-label, randomised, controlled pilot study with parallel assignment was performed. After obtaining board approval, 83 women <39 years old were recruited. Using blocked randomisation with randomly selected block sizes, the patients were divided into either the study group (n=43) or the control group (n=40).

Matching criteria were RIF, identified as ≥2 unsuccessful IVF attempts; good quality of previously transferred embryos according to the Gardner blastocyst classification; normal karyotype; and the absence of uterine factors of infertility. The age, BMI, and number of unsuccessful IVF attempts did not differ statistically between the groups. The mean number of unsuccessful IVF attempts was four. In the study group on the day of oocyte retrieval, the uterine flushing was collected using an insemination catheter, then marked and frozen. G-CSF concentration in uterine flushing was determined using ELISA and subsequently measurement per gram of protein.

The primary outcome of clinical pregnancy rate was analysed; there was no significant difference comparing between groups (chi-square: 0.018; $p>0.05$; continuity correction: 0.015). Thus, the method of collection, in this case uterine flushing, did not affect IVF outcome and can be used routinely. The G-CSF concentration in uterine flushing was significantly higher in women with clinical pregnancy with cut-off value of G-CSF being 0.151. The receiver operating characteristic curve showed a sensitivity of 87.5% and a specificity of 94.3%.

CONCLUSION

The method of collection of uterine flushing does not affect IVF cycle outcome and can be used routinely. According to obtained results, the evaluation of G-CSF level in uterine flushing can be considered a perspective, prognostic criterion for IVF cycle outcome with a sensitivity of 87.5% and specificity of 94.3%. However, as the study was a pilot, the number of recruited participants was limited. Thus, further investigation is needed. Nevertheless, these data prove the necessity for further research into the role of G-CSF in the implantation process and the need to consider the lack of G-CSF as a possible cause of recurrent implantation failure.

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Uterine Microbiota: A Role Beyond Infection

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The human body is colonised by many more bacteria than there are human cells. In addition to bacteria, there are other micro-organisms, although less abundant, such as viruses, fungi, microscopic eukaryotes, and archaea that contribute to the general microbiota. Recent research has highlighted the role of the microbiota in regulating human physiology in health and disease.[1,2]

Considering the abundance of microbiota, the totality of micro-organisms and their collective genetic material present in the human body, known as the human microbiome, has even been termed the second genome. The body's second genome offers new insights into the physiological condition and the dynamics between homeostasis and the pathogenesis of disease, with further extension to the promise of novel diagnostic and therapeutic approaches.

The female reproductive tract, specifically the vaginal milieu, has long been known to have an active microbiota. *Lactobacilli* are the cornerstone species in women of reproductive age, with studies in the USA determining that 3–4 community types of vaginal microbiota, out of 5 types identified, contain >90% *Lactobacillus*.[3] The lactic acid produced by the vaginal microbiota helps to maintain a low pH of 3.5–4.5, a major factor in limiting the growth of potentially harmful bacteria. Alterations in the vaginal microbiota play a role in common conditions, such as bacterial vaginosis, sexually transmitted diseases, urinary infections, and preterm birth.[4-7]

The uterus, on the other hand, was considered to be sterile until recently. The sterile womb paradigm, coined by Henry Tissier in 1900, was a commonly believed dogma that theorised that human infants develop within a sterile environment.[8] However, the assumption of a sterile, healthy uterus was challenged by multiple reports in the second part of the 20th century, which used culture-dependent methods to show bacterial colonisation varied from 0–90%.[9] With the advent of next-generation sequencing technologies in 2007, the belief that a healthy uterus is sterile has been revisited by recent studies, and it is now becoming clear that the uterus has micro-organisms with roles beyond infection. It is now acknowledged that only ~1% of bacteria are culturable,[10] and sequencing the unique bacterial 16S ribosomal RNA gene has resulted in an explosion in research to define microbial communities. Nevertheless, when applying next-generation sequencing we should be aware that the occurrence of a sole bacterial DNA is not consistent with the presence of living forms of the micro-organisms. In other

words, sequencing results do not enable us to distinguish between alive or dead bacteria within the sample, which is important information from a microbiological, clinical, and ecological point of view.

Recent studies have identified unique uterine microbiota that differ from that of the vagina;[9] however, the estimations of uterine bacterial load are estimated to be 100–10,000-times lower than that of the vaginal microbiome.[3,11] A pioneering study in the field analysed the microbiota along the female reproductive tract in 95 women of reproductive age.[3] The results showed that, contrary to the vaginal and cervix microbiota, *Lactobacillus* do not dominate and bacteria such as *Pseudomonas*, *Acinetobacter*, *Vagococcus*, and *Sphinogobium* constitute a notable fraction of the uterine microbiome (Figure 1). These bacteria grow in mildly alkaline conditions, contrasting to the *Lactobacillus*-dominated low pH environment of the vagina.

WHERE DO THEY COME FROM?

The microbiota in the uterus can originate from different sources and migrate to the uterus via various methods, including haematogenous spread, ascension from the vagina, via the sperm, and other methods (e.g., retrograde spread through the fallopian tubes or gynaecological procedures) (Figure 2).[9,12] Colonisation of specific bacteria via the haematogenous route has been shown in mice.[13] Bacterial spread through the bloodstream via either an oral route[14] or the gut[15] enables bacteria from mucosal sites, such as the oral cavity and gastrointestinal tract, to colonise distal mucosal sites, and this occurs during epithelial barrier breach.[13]

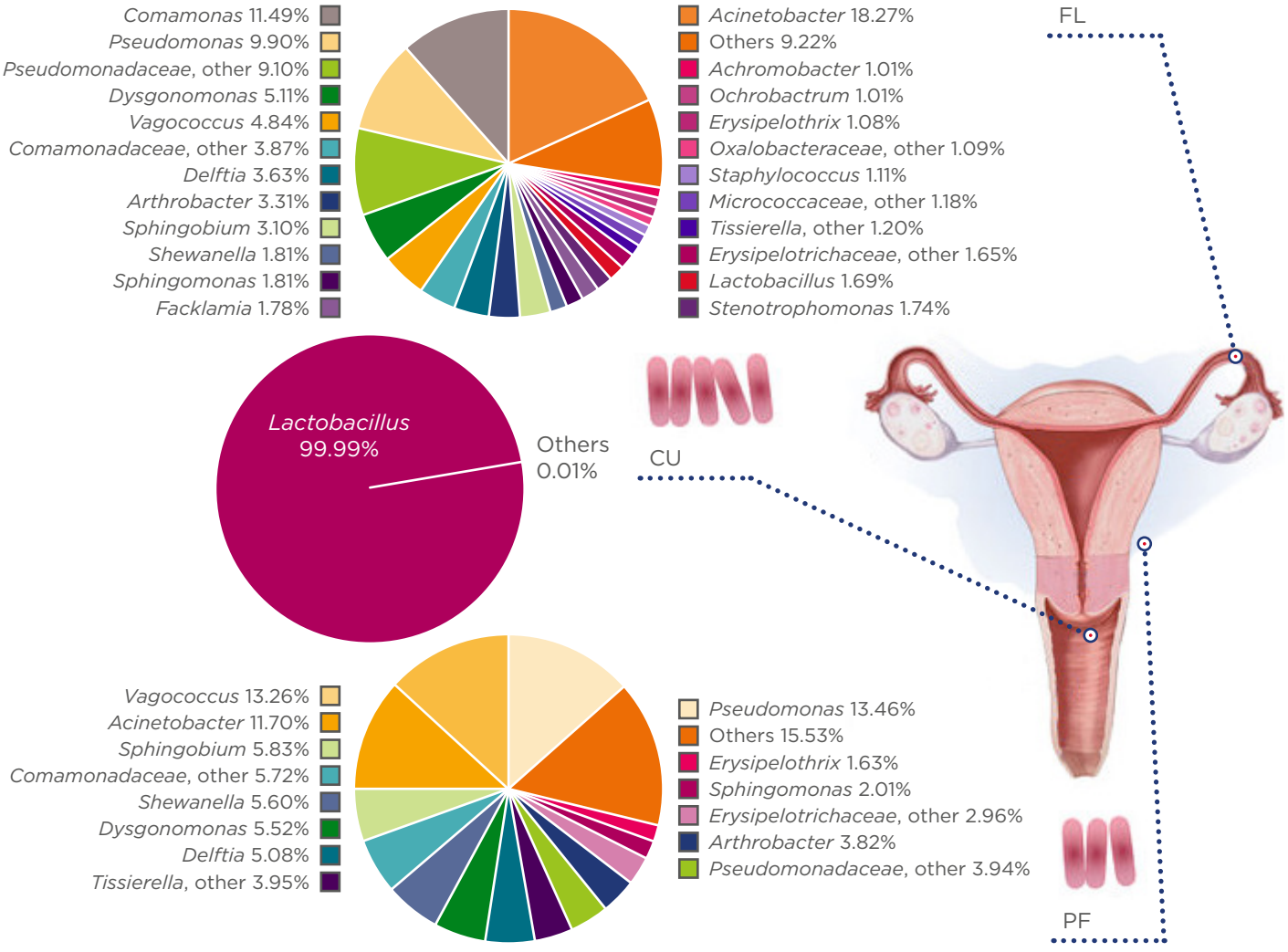

Figure 1: The microbiota continuum along the female reproductive tract.

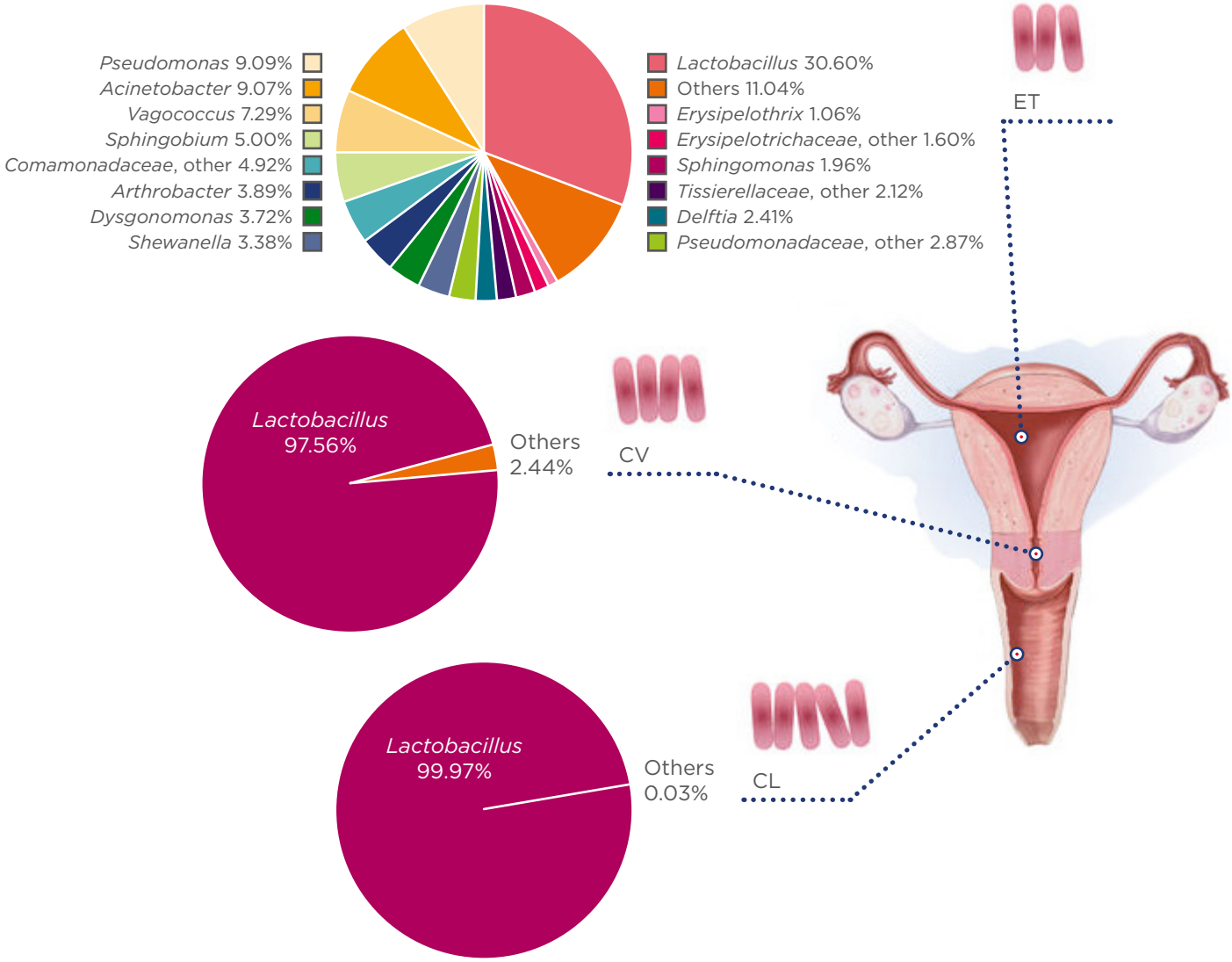

Figure 1 continued.

CL: lower third of vagina; CU: posterior fornix; CV: cervical mucus drawn from cervix; ET: endometrium/uterus; FL: fallopian tubes; PF: peritoneal fluid from the Pouch of Douglas.

Adapted with permission from Chen et al.[10] The figure is reproduced with permission from Nature Publishing Group.

Bacterial viability has been shown to be conserved during translocation through the blood, with intracellular dormancy being one way bacteria remain viable in the blood.[9]

Ascension of bacteria via the cervix has been well-established and is another highly probable source of bacterial transmission.[9] Other sources of uterine microbiota seeding may originate from assisted reproductive techniques, whereby bacteria from the vagina are introduced into the uterus (e.g., oocyte retrieval and embryo transfer) or during the placement of intrauterine contraceptive devices.[16,17] Additionally, there is a factor of sexual intercourse that can influence the uterine microbiota; it has been shown that sexual intercourse influences the vaginal microbiome[7,18] and, more specifically, that semen and vaginal microbiomes are in association.[19]

MICROBIOTA MAY MODULATE IMMUNITY IN THE UTERUS

Although the exact role and mechanisms of micro-organisms in the uterus are unknown, new studies have suggested that microbiota could be responsible for a receptive, fertile endometrium by influencing uterine immunity.[20]

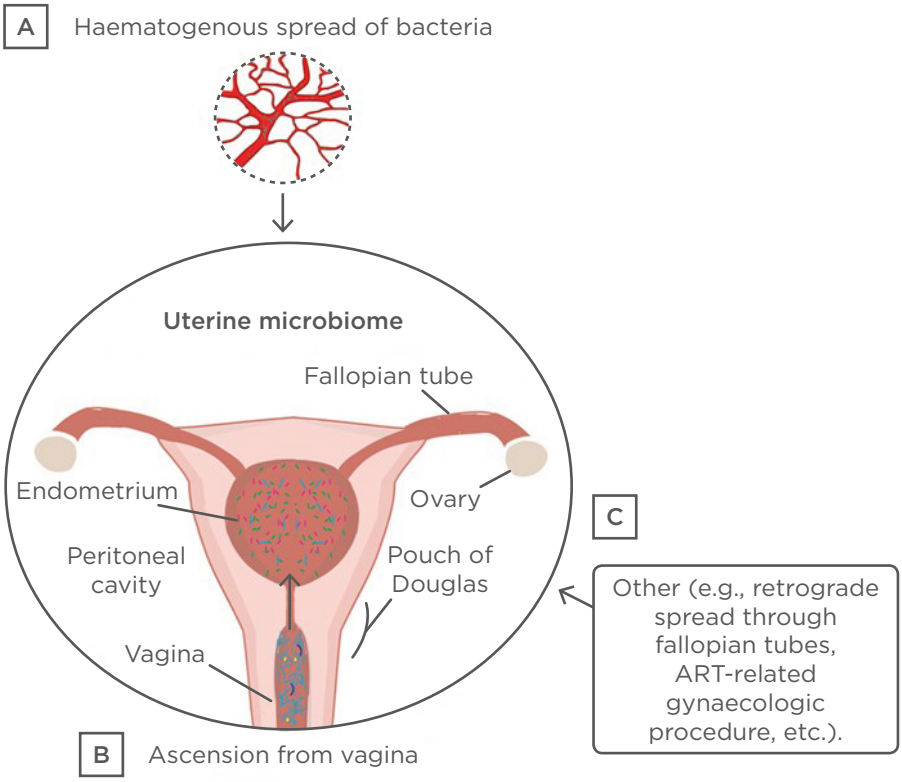

Figure 2: Established and putative bacterial transmission routes between uterine microbiota and distal sites.

ART: assisted reproductive technologies.

Adapted from Baker et al.[10] The figure is reproduced with permission from Nature Publishing Group.

The immune system is involved in all aspects of reproductive success, especially during the time of conception and in the peri-implantation period,[21] and it has been shown that local and systemic immunity is greatly influenced by microbiota.[22] Indeed, lessons learned from the gut microbiome suggest that the microbiota of the uterus could modulate immune cells involved in implantation and have implications for tissue morphology.[20] Microbiota can also be important in protection against infections by competing with invading pathogens in the uterus.[20]

UTERINE MICROBIOTA IN HEALTH AND DISEASE

To date, the main focus of uterine microbiota studies has been on the negative consequences of the presence of bacteria, and fewer studies have assessed the microbiome of a healthy uterus. The most abundant bacteria detected in the uterus belong to the phyla Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria.[9] However, the few studies analysing uterine microbiota in healthy asymptomatic women using next-generation sequencing show little consistency,[9] and further studies with larger sample sizes and including different ethnicities are needed to establish the normal or core uterine microbiota.

The presence of bacteria in the uterus has been associated with different gynaecological complications, including poor reproductive outcomes,[23-26] endometriosis,[10,24] dysfunctional menstrual bleeding,[27] and cancer;[28] nevertheless, a cause and effect relationship has not been clearly established. Uterine colonisation with bacterial vaginosis-associated bacteria has been suggested to promote carcinogenesis through microbiota-mediated pathophysiologic changes.[21,22] For example, a recent study[28] analysed the microbiome at various sites in the female reproductive tract and associated the presence of *Atopobium vaginae* and *Prophyromonas* species in the reproductive tract with cancer.

In women with endometriosis, the uterine microbiota composition has been shown to be different compared to healthy controls;[10,23] in these women, *Lactobacillaceae* are present in lower levels and *Streptococcaceae*, *Staphylococcaceae*, and *Enterobacteriaceae* species are enriched.[29]

A reduction in clinical pregnancy rates has been reported when bacteria were cultured from the *in vitro* fertilisation (IVF) catheter tip during assisted reproductive technology procedures,[30] which can seed the uterine microbiota and cause adverse reproductive and gynaecological outcomes by modulating the local microenvironment.[16] Other studies have detected a unique endometrial microbiota dominated by Bacteroides residing in the endometrium of women with different reproductive failures,[26] or no differences in endometrial microbiota at the moment of embryo transfer between infertile women with ongoing pregnancies and those without pregnancy.[23] In these women, Firmicutes (*Lactobacillus*) and Bacteroidetes (*Flavobacterium*) phyla represented the majority of the bacteria. The fact that Bacteroides regulate certain mechanisms in the gut that are relevant to the endometrium is intriguing, including mechanisms such as mucosal barrier reinforcement, epithelial cell maturation, and maintenance and interactions with the host immune system to control other bacteria.[31] Nevertheless, there is only one study[25] to date that has detected a statistical difference in microbiome profiles between successful and unsuccessful reproductive outcomes, rather than the mere presence of bacteria in the uterus. The study analysed 35 infertile women undergoing IVF (the biggest sample size analysed in this type of study) and the results demonstrated that non-*Lactobacillus*-dominated microbiota were associated with decreased implantation, pregnancy, and live birth rates among infertile women undergoing IVF.[25]

Larger sample sizes and standardised procedures to avoid or minimise vaginal cross-contamination are important aspects for future studies in determining the role of the uterine microbiota in reproductive health and disease.

CONCLUSION

The first studies of the uterine microbiota have highlighted significant changes in bacterial community compositions related to different gynaecological diseases, rates of IVF success, and risk of endometrial cancer. These pioneering studies provide a starting point for future research into the uterine microbiota to understand uterine physiology in health and disease, provide potentially effective clinical interventions for a variety of conditions, and have a positive impact on obstetric and gynaecological health.

In addition to the bacterial communities, other micro-organisms such as viruses, fungi, archaea, and microscopic eukaryotes exist in the uterine microflora that need to be investigated. Future studies should also clarify if the micro-organisms present in the uterus are residents that maintain homeostasis, tourists that are easily eliminated, or invaders that contribute to reproductive diseases. Furthermore, there is a need for studies investigating host–microbiota interactions and the physiologic and functional impact of the micro-organisms on the local endometrial microenvironment because these mechanisms may influence poor reproductive, obstetric, and gynaecological health outcomes.

As with any new research area, there are endless questions to be answered, but the uterine microbiota seems to have a high potential for providing additional knowledge of female reproductive functions and to predict and to improve its outcomes. It is time to consider micro-organisms not only as enemies but also as allies in reproductive medicine.

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Children After Cancer? Meeting Male Patients' Fertility Needs During Cancer Care

EDITOR'S PICK

My Editor's Pick for this edition focusses on an important hot topic. Young men (and, I would add, women) affected by cancer should always have the possibility to preserve their future fertility and receive appropriate counselling. Being responsible for the male gamete cryopreservation bank at the University Hospital of Careggi, Florence, Italy, I am aware that, all too often, consultants in a variety of fields forget to provide appropriate counselling to young male cancer patients regarding the possibility of fertility preservation before initiating any therapy that may affect testicular function. This article analyses the perceptions of all the stakeholders regarding fertility and cancer, remarking that banking spermatozoa reassures patients and helps them to face the battle against cancer. The article also analyses the main obstacles to fertility preservation, giving insights on how to surmount them. I found this article a very helpful read.

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Abstract

The prospect of cancer survivorship has changed significantly in the past decades. Cancer patients are now living longer and healthcare professionals are increasingly aware of the concerns of survivors with regard to quality of life. It is well known that psychological and social problems are created or exacerbated by cancer. At any stage of cancer survivorship, individuals may experience depression, a high sense of vulnerability, fear for the future, and other types of worries, such as changes in sexual function and reproductive ability. For many survivors, the ability to conceive and deliver a healthy baby is of paramount importance. However, in many circumstances, and for a variety of complex reasons, the importance of fertility is under-addressed and sometimes disregarded by the healthcare team. This article describes the significance of addressing fertility as a psychosocial need in male cancer patients, followed by a discussion on cancer patients' and family members' perceptions about the importance of fertility preservation. The authors also present practical strategies to improve the quality of services for cancer patients to address their fertility needs.

INFERTILITY AS A CAUSE OF PSYCHOSOCIAL DISTRESS IN MALE CANCER PATIENTS

Imagine if every healthcare professional would routinely ask "what matters to you?" when meeting with patients and their family members. In 2012, Barry and Edgman-Levitan[1] introduced the concept of asking "what matters to you?" in addition to "what is the matter?" to emphasise the need for healthcare providers to implement shared decision-making with patients and families regarding care plans. Using this patient-centred care approach, clinicians can inquire more deeply about what is really important to their patients and become more responsive to patients' values and preferences during the course of their illnesses.[1] In recent decades, a shift has occurred regarding cancer care, and research has clearly shown the importance of identifying, understanding, and addressing cancer patients' needs beyond those related to managing the cancer.[2,3] According to Zebrack et al.[4] and Gupta et al.,[5] three needs were identified as important to young adults with cancer: information on treatment and risk of recurrence of their specific malignancy, the effects of cancer treatment on fertility, and information on healthy diets and exercise during cancer treatment. Similarly, Klosky et al.[6] noted that good health and fertility were among the three top life goals in adolescent and young adult (AYA) cancer patients. In another study, 50% of men with cancer valued parenthood and expressed a wish to preserve their fertility.[7] Indeed, men who banked sperm prior to treatment felt more reassured and less worried about their fertility than patients who did not bank sperm, which helped them in the emotional battle against cancer.[8] Therefore, patients' concerns and uncertainty regarding fertility and parenthood are common forms of psychological stress, not only before or at the beginning of cancer treatment[9,10] but also during the post-therapy phase in the cancer survivorship trajectory, particularly for those cancer survivors who do not become parents.[7,11]

The National Comprehensive Cancer Network (NCCN) has defined distress as 'a multifactorial unpleasant emotional experience of a psychological (i.e., cognitive, behavioural, emotional), social, and/or spiritual nature that may interfere with the ability to cope effectively with cancer, its physical symptoms, and its treatment'.[12] The NCCN also state that all patients with cancer and their families should be able to expect and receive cancer care that ensures the delivery of appropriate psychosocial health services, which are recognised as an essential component of quality of care.[12-14] Similarly, the American Society of Clinical Oncology (ASCO),[15] the American Academy of Pediatrics (AAP),[16] the European Society for Medical Oncology (ESMO),[17] and the American Society for Reproductive Medicine (ASRM)[18] have recommended clinicians to discuss with all newly diagnosed cancer patients the potential impact of cancer treatments on future fertility and to present options for fertility preservation. Despite the recognition by multiple professional organisations and medical groups that fertility preservation is an essential part of comprehensive cancer care, recent studies have indicated that proper pretreatment fertility counselling was disseminated to only a minority of newly diagnosed cancer patients.[19,20]

STAKEHOLDERS' PERCEPTIONS OF FERTILITY AND CANCER

Why then is fertility not always part of the conversation between clinicians and patients? How can healthcare providers make a positive healthcare experience for male patients with cancer as they transition from patient to survivor? Although there is no simple, universal answer or solution to these important questions, one approach is to first evaluate and understand stakeholders' perceptions about fertility. These stakeholders include cancer patients and their family members, as well as clinicians. While the bulk of the existing literature has focussed on cancer survivors' needs after their treatments, recent studies have explored patients' needs at the time of diagnosis, especially those of AYA cancer patients. Male AYA are as susceptible to the adverse effects of cancer treatment as fully mature men and are considered a vulnerable population because cancer diagnosis and treatment can be particularly disruptive to their social maturation, a process by which young people develop self-identity and social awareness that will guide them throughout their lives.[21] This unique cancer

population, which includes patients between the ages of 15 and 39 years, encounters challenges that differ from those of children and older adults with cancer.[2,22] Feeling different from their peers as a result of the cancer diagnosis is a source of stress for AYA cancer patients, who often report feelings of isolation. These feelings could be due to missing out on important social activities, such as sports and school dances, or experiencing stigma and unfair treatment due to changes in their appearance, adding to the fact that their peers often have little familiarity with illness and do not know how to extend their support to a friend with cancer.[2]

Since perceptions may differ from the time of cancer diagnosis to the various stages along the trajectory of survivorship, it is important to analyse all of these points of view. Thus, the different stakeholders' opinions regarding fertility of AYA cancer patients are briefly summarised in the following sections.

Adolescent and Young Adult Cancer Patients' Points of View at Time of Diagnosis: Patients diagnosed with cancer are often worried about not being able to have children and have highlighted the need for access to high-quality information about sexuality and fertility.[23]

Adolescent and Young Adult Cancer Survivors' Points of View: One of the various themes discussed by AYA cancer survivors is the fact that infertility often comes as a surprise to cancer survivors, and patients have voiced regret about fertility risks not being addressed at the time of cancer diagnosis.[23] Cancer experience increases the value many patients place on family, therefore, increasing their desire to have children.[3,6] Moreover, cancer survivors have expressed desire that oncology healthcare providers and fertility specialists have a proactive involvement with patient fertility.

Cancer Patients' Parents' Points of View at Time of Diagnosis: Parents play a key role in the co-ordination and execution of care for adolescents. For many parents of cancer patients, the prompt initiation of treatment, rather than fertility preservation, is the primary concern. Moreover, they often do not feel at ease or equipped to discuss the subject of fertility with their children following a new cancer diagnosis. Nevertheless, many parents are interested in receiving information related to fertility and are concerned about the negative consequences of infertility in regard to its impact on relationships.[24]

Cancer Survivors' Parents' Points of View: Many parents have expressed similar regrets regarding fertility, as well as a strong sense of guilt.[23] In contrast to parents of newly diagnosed cancer patients, parents of cancer survivors reported that their children should have been involved in fertility discussions regardless of the patient's age at diagnosis and expressed that discussing fertility issues with the oncology healthcare provider would have fostered a feeling of optimism.[23]

Oncologists' Points of View: Practitioners have expressed concern about the inadequacy of time for conversations regarding fertility preservation at the time of cancer diagnosis, since there is often a short time period between cancer diagnosis and initiation of treatment, and they perceive that fertility preservation could potentially delay cancer treatment. Furthermore, many oncologists also feel unequipped and uneasy discussing fertility preservation with patients due to their lack of knowledge on the effect of fertility and cancer, the difficulty finding convenient fertility clinics for referral, their perceptions on ethical issues regarding conversations with minors, and the uncertainty on how to approach the topic of fertility preservation in patients with poor prognosis or developmental delay.[25-27] Practitioners have also expressed concerns regarding the out-of-pocket cost to patients and the possible resulting inequity of service delivery based on income.[27]

Taken together, infertility is a cause of concern and anxiety among cancer patients and their parents. Contrary to the common assumption of clinicians and parents that cancer patients are too overwhelmed to handle information regarding risks of infertility due to cancer treatments, patients want to receive information on their current reproductive status and future reproductive risks.[23,28,29] Moreover, cancer survivors and their parents share a sense of regret for not considering fertility preservation at the time of cancer diagnosis. Discussing infertility with AYA cancer patients during diagnosis is therefore needed to prevent the issue from becoming a silent concern and to minimise negative future effects.

These perceptions are not unique to AYA cancer patients; fertility is important to all males newly diagnosed with cancer, regardless of age and situation. For example, older, married, or homosexual men who are not informed about the option of preserving sperm often manifest high levels of confusion and anger towards healthcare professionals.[30] With recent dramatic progress in the access to assisted reproductive technologies, open and timely communication followed by a referral process from the oncology team to the fertility specialists is essential to ensure quality of care to all males newly diagnosed with cancer and to minimise unnecessary distress and litigation. A study by García et al.[31] demonstrated that male cancer survivors who previously cryopreserved sperm and later on in life sought assisted reproduction using their frozen sperm, presented comparable reproductive outcomes to a non-cancer population undergoing *in vitro* fertilisation (IVF) treatment; this finding supports the notion that sperm banking or cryopreservation for cancer patients is a highly valued service that should be encouraged for all male cancer patients prior to gonadotoxic cancer treatment.[31]

Finally, as previously mentioned, for young cancer patients, cost could be a barrier to pursue fertility preservation.[27,32] Moreover, since cryopreserved sperm are of finite quantity, the more sessions of sperm cryopreservation carried out the lower the risk of sperm quantity limiting the success of future assisted reproduction. In 2010, the province of Quebec, Canada became the first North American jurisdiction to offer full financial coverage, through provincially funded Medicare programme, for 5 years of sperm cryopreservation and storage for cancer patients. It has been shown that there has been a significant increase in the number of sperm banking sessions per cancer patient after the provincial implementation of the sperm banking coverage, when the practice pattern of oncologists was constant,[33] suggesting that once cancer patients are aware of the option of freezing sperm, and when cost is no longer a barrier, they are interested in banking sperm and quality of care is improved. Based on the research described, it is important that oncology care providers, who are at the front-line to counsel cancer patients, are aware of the availability, accessibility, and reproductive success of fertility preservation.

IMPLEMENTATION OF SUCCESSFUL FERTILITY PRESERVATION PROGRAMMES

What are the challenges encountered when establishing a successful fertility preservation programme? Although sperm freezing is a relatively accessible clinical procedure that is accepted as part of the standard of care when managing male cancer patients, there are several challenges inherent to the decision-making process in fertility preservation. Firstly, since sperm banking is most effective before cancer treatment begins, gathering and assimilation of information about fertility preservation need to be accomplished within a relatively short timeframe, usually within days but often hours.[15] Secondly, sperm banking requires the involvement of a multidisciplinary team, such as oncology healthcare professionals, urologists, mental health professionals, and fertility specialists, with continuous interaction, open communication, and knowledge-sharing being essential to bridge the gap between specialities. Thirdly, as previously mentioned, cost may be a significant financial burden for patients at cancer diagnosis. Therefore, the establishment of a successful fertility preservation programme should be equipped to address many of these potential hurdles regarding decision-making in fertility preservation.

The goal of a clinical fertility preservation programme is to help patients and their physicians evaluate the impact of cancer treatment on fertility and to facilitate fertility preservation options in a timely manner.[34] When developing a successful fertility preservation programme, there are several key considerations, some of which are outlined in the following sections.

Institutional Commitment for Fertility Preservation: As with any other programme, to be successful it is essential that the organisation is supportive. For instance, in 2005 Fertile Hope/LIVESTRONG launched the Centers of Excellence (COE) programme to recognise cancer centres that had made an institutional commitment to meet their patients' reproductive needs in a deliberate, methodical way.[35] Among the suggested criteria to support institutional commitment are the issuance of formal hospital-wide policy and the

description of fertility preservation discussions as part of standard operating procedures on the hospital's intranet.[36]

An Efficient Fertility Preservation Referral Process: It is important to recognise that patients are strongly influenced by the messages they receive from their oncologist and may be more likely to seek fertility preservation services if the clinician introduces this topic as a legitimate concern.[28,37] Therefore, it is essential for clinicians to feel comfortable initiating fertility discussions with their patients. In order to facilitate this task, clinicians need to know where and how to refer interested patients to reproductive specialists and be educated about the topic. It is important to cultivate a strong relationship between the oncology and fertility teams to facilitate a fertility preservation referral process and bridge the gap between these two specialities.

Professional Education on Fertility Preservation: As previously mentioned, for patients to receive proper fertility preservation counselling, oncology healthcare providers need to feel comfortable conveying this information to patients. For this purpose, educational presentations could be delivered effectively through various, continued, medical education channels, including ground rounds, staff orientations, case presentations, and medical conferences.[38-40] Also, clinicians should be provided with access to resources at the time they encounter the patients. Printed resources, such as posters and brochures, as well as an internal website on fertility and cancer treatments, can aid clinicians with fertility counselling.

Patient Education on Fertility Preservation: The goal of patient education should be to provide helpful information to patients about the risks of cancer treatment on fertility. By increasing their knowledge, clinicians will empower patients to participate in the decision-making process, thus engaging them to adopt an active role in managing their care.[40,41]

In the USA and Canada, several institutions have developed strategies to formalise oncofertility programmes, with the goal of increasing the number of men receiving fertility preservation consultations, leading to larger percentages of patients opting to cryopreserve sperm.[34,42-44] These strategies include:

- The implementation of a standard process through a continuous process improvement approach, which was developed at Seattle Children's Hospital, Seattle, Washington, USA, where patient and staff education material was created.[43]
- A referral system incorporated at the Moffitt Cancer Center, Tampa, Florida, USA, in which, after educating clinicians on fertility preservation and generating a brochure for patients, an electronic system prompted the physician to distribute a brochure to interested patients on fertility during their initial visit.[42]
- An alert on medical electronic records that reminds the treating physician to discuss fertility preservation options with new cancer patients at Northwestern University, Evanston, Illinois, USA.[44]
- Resources for patients and clinicians, education of clinicians, and a consultation service incorporated in a comprehensive cancer and fertility programme at the Memorial Sloan Kettering Cancer Center, New York City, New York, USA.[41]
- The designation of a dedicated clinical nurse for pre and post-therapy counselling on infertility risks included in the AYA programme at the Princess Margaret Cancer Centre, Toronto, Canada.[45]
- The development of a suite of educational tools and materials for patients, such as brochures, frequently asked questions, and videos posted at the MUHC Reproductive Center's website, as well as educational talks on fertility preservation for health care providers at the McGill University Health Center, Jews General Hospital and St. Mary's Hospital Center, Montreal, Canada.[46]

CONCLUSION

In conclusion, research has clearly demonstrated that cancer patients wish to receive information about fertility, that a large proportion of cancer patients informed about the infertility risk of cancer therapy choose to freeze their sperm, and that professional guidelines recognise the value of future fertility of cancer survivors. However, there are still persistent obstacles for oncology healthcare professionals to initiate a discussion on the reproductive risks and management options with their

patients. Both patients and clinicians should adopt a proactive approach towards fertility preservation, and the creation of institutional policies addressing fertility as a psychosocial need should be an integral part of cancer care.

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A Powerful and Universal Preimplantation Genetic Diagnosis Protocol for Cystic Fibrosis

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Abstract

Background: Cystic fibrosis (CF) is one of the most common indications of preimplantation genetic diagnosis (PGD) for monogenic disorders worldwide.

Aims: The aim of this article was to report a universal and powerful assay easily applicable to all couples requesting PGD for CF irrespective of the *CFTR* variants involved, in line with recently published CF-PGD guidelines.

Results: A multiplex PCR protocol was developed including the study of the c.1521_1523del mutation with 12 closely linked polymorphic markers. Preliminary workup was performed for 53 couples and the protocol was clinically applied to 31 couples. All couples were informative for 7–12 markers. Of the 31 couples who initiated a PGD stimulation cycle, 17 couples had a baby. Therefore, the take-home baby rate was 60.7% per couple with an embryo transfer (17 out of 28 couples).

Conclusion: This robust, simple, and reliable procedure should allow any couple at risk of transmitting CF to enrol in a PGD programme.

INTRODUCTION

Preimplantation genetic diagnosis (PGD) was first reported in the 1990s as an alternative option to prenatal diagnosis for couples at risk of transmitting a severe monogenic disease or chromosomal disorder to their children.[1] The procedure is based on genetic analysis of embryonic cells biopsied from preimplantation embryos obtained through *in vitro* fertilisation (IVF) techniques. Only embryos free of the disease under investigation are transferred to the mother's uterus to initiate a pregnancy. PGD in France is strictly regulated by law (only the parental genetic risk can be studied; concomitant aneuploidy screening is not allowed) and is a rare example of an entirely free-of-charge service within the public health organisational framework, thus providing equal access to PGD.[2]

Cystic fibrosis (CF, OMIM# 219700), a frequent and lethal genetic condition, was the first monogenic disorder to be studied in a PGD clinical case.[3] Thereafter, different PGD protocols have been published for CF, ranging from the study of the c.1521_1523del mutation (p.Phe508del) alone[4] to more generic strategies based on the study of polymorphic-linked microsatellite markers with or without direct mutation analysis[5,6] or karyomapping through analysis of SNP genotypes on microarrays.[7] In order to harmonise protocols and procedures for CF-PGD across PGD centres in Europe, specific best practice guidelines have been recently published.[8]

In line with these guidelines, this paper describes a PGD protocol for CF that presents a notable improvement compared to previously published methods,[4-6] as it is now based upon the study of 12 polymorphic markers within or close to the CF transmembrane conductance regulator (*CFTR*, OMIM# 602421) gene, associated with the direct analysis of the c.1521_1523del mutation.

MATERIALS AND METHODS

Microsatellite Marker Selection

The 9-plex PCR haplotyping approach used previously[6] was implemented with the analysis of four additional short tandem repeats (STR) across and flanking the *CFTR* gene: D7S633 at ~100 kb upstream to the *CFTR* gene, IVS9TAAA in intron 10, and both CFTRSTR30AC and CFTRSTR15CA microsatellites at ~100 kb and 200 kb downstream to the *CFTR* gene, respectively.[9] All primers were carefully designed and fluorescently labelled to obtain amplicons with different lengths (Table 1) after separation by capillary electrophoresis (Figure 1).

Table 1: Overview of the four additional single tandem repeat markers and the newly designed IVS10CA marker used in the 13-plex preimplantation genetic diagnosis protocol for cystic fibrosis. Their chromosome position, location within the gene or distance to the *CFTR* gene, type of single tandem repeat and sequence primers, heterozygosity rates, and number of alleles are indicated.

Marker (location on chromosome 7*)	Type	Sequence primers	Distance to the *CFTR* gene	Heterozygosity rate (%)†	Number of alleles
D7S633 (117,370,706-117,370,903)	(CA)n	F: Hex-CAGTGAGCCTCGCATCACTG R: GTTGACAAGTGTATTAGATGACC	~100 kb	76.0	14
IVS9TAAA (117,558,332-117,558,620)	(TAAA)n	F: Hex-TTTTCGAGGTTAGGAGATCAAGAC R: AGGAGGTAGCAGAGGAAGAAAAAG	intron 10	79.1	6
IVS10CA (117,566,128-117,566,329)	(CA)n	F: Fam-TGGACATCTGAAACAGGTATTTG R: CCAAGTAGCTTGGACTACAAACG	intron 11	92.8	11
CFTRSTR30AC (117,773,551-117,773,782)	(CA)n	F: Hex-TACTGCAGGAGCCACTGTTG R: TGCCCTTTTCTCACTTCCTC	~100 kb	87.6	15
CFTRSTR15CA (117,871,615-117,871,936)	(CA)n	F: Fam-CGTCCTGGGTTTTGTTTACG R: ATGATTTGGTGCCTTCATCC	~200 kb	90.7	23

*According to UCSC GRCh38/hg38, December 2013;[10] †calculated by typing 129 unrelated individuals.

F: forward; R: reverse.

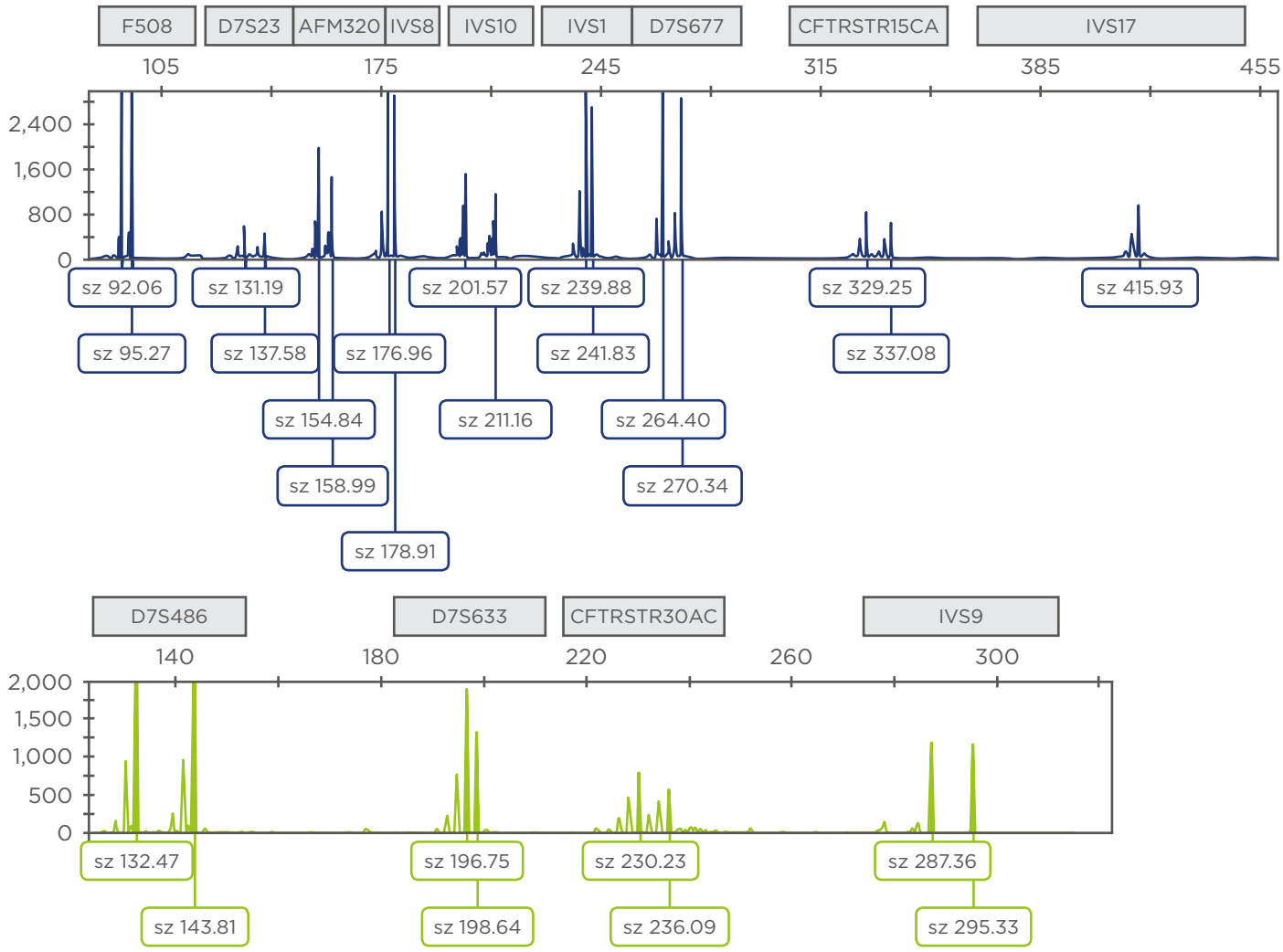

Figure 1: Electropherogram obtained using the generic 13-plex PCR protocol for cystic fibrosis.

Examples of the amplicons are shown for each STR (identified by the squares above the electropherogram). Fam labelled amplicons are shown in blue (top) and Hex labelled amplicons are shown in green (bottom).

STR: short tandem repeats; sz: size.

Primers for IVS10CA in intron 11 had to be modified to improve electrophoretic peak pattern in this newly designed single-tube PCR protocol (Table 1).

To estimate heterozygosity rates and to assess informativity for each marker in PGD couples, careful preliminary workup using 13-plex PCR was performed on 100 ng genomic DNA samples from 129 unrelated individuals heterozygous for one severe or large spectrum *CFTR* gene mutation, including 53 couples enrolled in our PGD programme for CF and 23 individuals referred to our laboratory for CF diagnosis.

Single Cell Multiplex PCR

Before clinical application, to set up the optimal PCR conditions for co-amplification of the 12 STR markers together with the p.Phe508del, >300 single lymphocytes were isolated from different individuals and lysed.[6] Reaction mixes were added to the lysed samples to produce a 30 µL final sample. The PCR sample contained 15 µL of 2X Qiagen multiplex PCR master mix and 0.17 µM each of the pairs of primers for p.Phe508del, D7S633, and CFTRSTR30AC; 0.34 µM each of the pairs of primers for D7S486, AFM320vb5, D7S677, D7S23, and IVS10CA; 0.5 µM each of the pairs of primers for IVS1CA and IVS9TAAA; 0.67 µM

each of the pairs of primers for IVS8CA and CFTRSTR15CA; and 0.84 µM each of the pairs of primers for IVS17bTA/CA. Thermal cycling consisted of an initial denaturation step at 95°C for 15 minutes, followed by 40 cycles at 94°C for 30 seconds, 58°C for 90 seconds, and 72°C for 60 seconds, then a final extension step at 60°C for 30 minutes. Of the amplified products, 1 µL was run on an ABI 3130XL DNA sequencer and the results were analysed using Genemapper v4.0 software (Applied Biosystems, Foster City, California, USA). For couples carrying variants other than p.Phe508del, informative markers can also be co-amplified with mutation-containing *CFTR* amplicons to combine mutation detection using a minisequencing approach and linkage analysis.[11]

The PCR protocols were identical for both single lymphocytes (pre-PGD workup) and biopsied blastomeres from preimplantation embryos (PGD cycles). The IVF part of the PGD procedure has been detailed elsewhere.[12]

RESULTS

Heterozygosity Rates and Informativity

Heterozygosity rates for the four newly designed microsatellite markers D7S633, IVS9TAAA, CFTRSTR30AC, and CFTRSTR15CA ranged from 76.0–90.7% (Table 1). Among the 53 couples who had a familial pre-PGD workup for CF using the 13-plex PCR protocol, the number of informative markers ranged from 7–12. For the eight couples who displayed only 1–3 fully informative markers using our previously described protocol,[6] the study of the four newly designed STR yielded a better informativity with at least one additional fully informative marker.

Single cell amplification and allele drop out (i.e., the random non-amplification or detection of one allele in a heterozygous sample) rates for the different sequences were within the ranges established by the European Society of Human Reproduction and Embryology (ESHRE) PGD consortium best practice guidelines.[13] We therefore considered that the updated protocol, detailed in this report, fulfilled the criteria of a reliable clinical PGD method.

Preimplantation Genetic Diagnosis Cycles

From July 2014–December 2017, 31 couples initiated at least one PGD stimulation cycle for CF, including 6 couples with a 50% risk of having a CF-affected child (one CF-affected member, the other being a carrier of a severe or large spectrum *CFTR* mutation) and 25 couples with a 25% risk (both partners heterozygous for a severe or large spectrum mutation) (Table 2).

Table 2: Overview of the clinical application of the improved cystic fibrosis preimplantation genetic diagnosis protocol to 31 couples.

	Risk of having a CF-affected child		Total
	50% risk*	25% risk†	
Couples with stimulating cycle	6	25	31
Couples with embryo transfer	5	23	28
HCG positive (>1,000 mIU/mL)	5	16	21
Clinical pregnancies (FHB+)	4	15	19
Singleton pregnancies	3	14‡	17
Twin pregnancies	1	1§	2
Healthy children born	5	13	18

*One member of the couple affected with CF, the other heterozygous for a CF-causing or a large spectrum *CFTR* mutation; †both partners heterozygous for a CF-causing or a large spectrum CFTR mutation; ‡one termination of pregnancy (25 weeks of pregnancy); §spontaneous miscarriage (8 weeks of pregnancy).

CF: cystic fibrosis; FHB+: positive fetal heartbeat; HCG: human chorionic gonadotropin.

Twenty-eight couples (90%) had one or two unaffected embryo(s) transferred and 19 pregnancies (19 couples) with a positive fetal heartbeat were achieved (Table 2). A total of 17 deliveries occurred with 18 healthy babies born.

Two adverse outcomes were recorded, including a spontaneous twin pregnancy miscarriage (at 8 weeks) and a termination of pregnancy not related to the PGD indication (at 25 weeks). None of the newborns were diagnosed with CF following testing through the French national newborn screening programme. Therefore, the take-home baby rate is 60.7% per couple with an embryo transfer (17 out of 28 couples).

DISCUSSION

CF is the most common indication for a monogenic disorder at our centre, and one of the most common indications of PGD for single gene disorders reported in the last ESHRE PGD consortium data collection.[14] Because >2,000 different genetic variants have been described in the *CFTR* gene,[15] developing and optimising specific single cell PCR tests for each variant is impossible. Novel generic haplotyping technologies, such as karyomapping using SNP arrays,[7] are now commercially available for clinical use in PGD and are claimed to be applicable to numerous genotype combinations without prior extensive case-specific workup. However, the cost of the platform is high, the interpretation sophisticated, and the informativity of the biallelic markers may be limited in some cases. PCR-based multiplex assays using informative polyallelic STR markers are still largely used,[14] as they represent the simplest strategy allowing the application of a unique genotyping PGD protocol for all couples at risk of transmitting the same monogenic disorder.[16]

Compared to the 9-plex PCR protocol, which we have previously published, the high number of DNA sequences studied allowed us to obtain conclusive results for all embryos with positive amplification signals, increasing the opportunity to identify unaffected embryos and to achieve a pregnancy. The efficiency and accuracy of our 13-plex PGD protocol is evidenced by the take-home baby rate of 60.7% per couple with embryo transfer. The robust, simple, reliable, and low-cost procedure described in this report should allow the rapid enrolment of any couple at risk of transmitting CF in a PGD programme.

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Reproductive Medicine Involving Mitochondrial DNA Modification: Evolution, Legality, and Ethics

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Abstract

Human oocytes have an abundance of mitochondria that have their own genome. Mitochondrial functions are exerted through evolutionarily-developed interactions between the nucleus and mitochondria. Since 1996, fertility clinics have practiced various types of germline mitochondrial DNA (mtDNA) modification that alter the composition of mtDNA copies in oocytes or zygotes using micromanipulation. Experimental reproductive medicine has primarily intended to treat intractable infertility and has been used to prevent the maternal transmission of a pathogenic mtDNA mutation to offspring. In some cases, it has helped parents have a healthy genetically-related child; in others, it has resulted in miscarriages, aneuploid fetuses, or developmental disorders in the offspring. Adverse events have raised ethical controversy, leading to restrictive or prohibitive policies in the USA and China. Conversely, the UK recently became the first nation to explicitly permit two types of germline mtDNA modification (termed mitochondrial donation) for the sole purpose of preventing serious mitochondrial disease in offspring. The aim of this review is three-fold: first, to reshape the medical concept and evolution of germline mtDNA modification, while revisiting 14 clinical cases. Second, to analyse the legality of mtDNA modification, focussing on 16 Western countries. Finally, to consider the ethical aspects, including permissible cases, reproductive options, use of preimplantation and prenatal testing, and the humane follow-up of resultant children. The clinical use of germline mtDNA modification will likely become legal, at least for use in preventative medicine, in some countries. However, the potential clinical, ethical, and evolutionary implications mean that caution is required when considering its wider application.

INTRODUCTION

The majority of human cells have two genomes: nuclear DNA (nDNA), with approximately 24,000 protein-coding genes, and mitochondrial DNA (mtDNA), with only 13 protein-coding genes. Mitochondria are small organelles that exist in the cytoplasm and are involved in various cellular functions. The production of ATP through the respiratory chain is one of the most important functions of the organelles.

Mitochondrial functions are exerted through the co-ordinated expression of genes in mtDNA and nDNA, which have become highly specific over evolutionary time. Regarding human mtDNA, a spermatozoon has 100–1,500 copies of the organelle genome, whereas a mature oocyte has as many as 200,000–300,000 copies of mtDNA.[1] Paternal mitochondria are specifically digested after fertilisation; as a result, only maternal mtDNA is transferred to the offspring. Mutations to the 13 protein-coding mtDNA genes have been linked to various forms of human mitochondrial disease.[2] Although *POLG* in the nDNA, which encodes the catalytic subunit of mitochondrial DNA polymerase, has been suggested to be associated with infertility, mtDNA genes that only cause infertility remain elusive.[3,4]

From the 1980s to the early 2000s, rodent experiments have demonstrated the feasibility of altering the cytoplasm of oocytes (ooplasm) by cytoplasmic transfer. Soon after, it was demonstrated that the cytoplasm of embryos can be largely replaced by transferring a karyoplast (nuclei [or a nucleus] with a plasma membrane containing a small amount of cytoplasm) to a different enucleated zygote.[5-7] Such outcomes led to the development of reproductive medicine involving a cytoplasmic or karyoplast transfer that alters the composition of mtDNA copies in oocytes or zygotes. In 1996, a clinic in the USA initiated ooplasmic transfer (OT), and reported the birth of a baby in 1997; this is believed to be the first case of human germline genetic modification.[8,9] Subsequently, some OT cases have helped prospective parents have a genetically-related child, whereas others have resulted in miscarriages, aneuploid fetuses, and the onset of a developmental disorder in the offspring.[10,11] In 2003, a collaboration between a Chinese group and a team from the USA reported the first pronuclear transfer (PNT), which was performed with the intention of largely replacing the cytoplasm of a patient's zygote with that of a donor zygote.[12] The PNT performed in China led to a triplet pregnancy; however, two fetuses died after selective fetal reduction. Such adverse events have led to restrictive or prohibitive regulatory policies in the USA and China.[13] Conversely, in 2015, the UK legalised PNT and maternal spindle transfer (MST), which can largely replace ooplasm, for the sole purpose of preventing serious mitochondrial disease in offspring.[14] In 2017, the first MST procedure performed by researchers from the USA and Mexico led to the birth of a healthy baby.[15]

With the current climate concerning mtDNA modification in mind, this article first reviews the medical concept and evolution of germline mtDNA modification, while revisiting 14 clinical cases. Next, the legality of the procedures is analysed, focussing on 16 Western countries, because an international treaty in the biomedical field was established in Europe.[16] Furthermore, ethical aspects are considered regarding permissible cases, reproductive options, the use of preimplantation genetic diagnosis (PGD), and prenatal testing and humane follow-up of resultant children.

MEDICAL CONCEPT AND EVOLUTION

Table 1 shows 14 clinical cases of germline mtDNA modification that have been performed in nine countries. Eleven reports were published from 1997–2003. The remaining three reports were published within the last 3 years, after a decade-long period without relevant publications.

The Beginning of Germline mtDNA Modification

In 1996, a USA clinic initiated a clinical study of OT, in which 5–15% of ooplasm aspirated from mature oocytes donated by fertile women was injected into mature oocytes of infertile patients, along with a spermatozoon.[11] The subjects included 33 infertile women who had experienced repeated implantation failure and poor embryo development after *in vitro* fertilisation (IVF).[17] Based on a hypothesis that IVF failures could be due to cytoplasmic deficiency rather than aneuploidy in nDNA, the study intended to enhance the developmental potential of the patient's embryos. In 1997, a girl was born via OT (Table 1).[8] mtDNA typing showed sustained heteroplasmy representing both donor and recipient mtDNA in the clinical specimen, suggesting that heteroplasmic mitochondrial populations persist and may be replicated during development (Table 1).[9]

Table 1: Clinical implementations of germline mitochondrial DNA modification.

Report	Place of practice	Procedure	Origin of mitochondria for transfer	Manipulation	Patient/s	Clinical consequences	Remarks
Cohen et al.,[8] 1997	USA	OT	Donor oocytes	Cytoplasmic transfer	A 39-year-old woman with poor ovarian reserve, 6.5 years of infertility, and four IVF or ICSI failures.	A girl was born from a singleton pregnancy.	Inadequate embryo development was observed.
Cohen et al.,[18] 1998	USA	OT	Donor oocytes	Cytoplasmic transfer	Seven couples with quality-compromised oocytes and embryos.	Electrofusion of ooplasm ended in no pregnancies in three couples. Direct injection led to three pregnancies in three couples (one live birth, one miscarriage, one ongoing pregnancy at time of study publication); and no pregnancies in two couples with male infertility.	Direct injection of ooplasm into oocytes may be better than electrofusion.
Huang et al.,[20] 1999	Taiwan	OT	Donor tripronucleate zygotes	Cytoplasmic transfer	Nine women with >5 IVF or ICSI failures (32–42 years old).	Five healthy infants were born from four recipients. No pregnancies in the remaining five recipients.	<30-year-old women who underwent IVF donated tripronucleate zygotes.
Lanzendorf et al.,[19] 1999	USA	OT	Frozen-thawed donor oocytes	Cytoplasmic transfer	Three women of advanced maternal age (43, 47, and 47 years old), and one woman (35 years old) with poor embryo quality on six IVF cycles.	Three women of advanced maternal age did not achieve pregnancy. A 35-year-old woman delivered healthy male and female infants.	Live births happened using oocytes injected with cytoplasm from frozen-thawed oocytes of a 30-year-old donor.
Tzeng et al.,[22] 2001	Taiwan	AGCMT	Autologous granular cell	Mitochondrial transfer	Patients aged >38 years, recurrent implantation failure, prolonged unexplained infertility, poor fertilisation rate, or compromised embryo quality in previous cycles.	Three clinical pregnancies. One of the cases, a twin pregnancy in a 36-year-old patient with infertility of 9 years, showed normal 46XX and 46XY.	Conference paper, not peer-reviewed article. 500–5,000 mitochondria were injected in each oocyte.
Levron et al. personal communication, introduced in Barritt et al.,[11] 2001	Israel	OT	Donour oocytes	Cytoplasmic transfer	Not shown.	15 treatments led to six live births from five pregnancies.	Personal communication.
Dale et al.,[21] 2001	Italy	OT	Donor oocytes	Cytoplasmic transfer	A couple with 7 years of infertility (a 32-year-old woman and a 35-year-old man).	Healthy twins were born from a twin pregnancy.	High level of embryo fragmentation and poor development was observed.
Barritt et al.,[11] 2001	USA	OT	Donor oocytes	Cytoplasmic transfer	See remarks column.	One singleton pregnancy ended in a miscarriage (Turner syndrome: 45,X0). Another fetus (45,X0) in a twin pregnancy was selectively reduced. A male of a male–female twin was diagnosed with pervasive developmental disorder.	This paper was an analysis of the worst cases from Cohen et al.,[8] 1997 and Cohen et al.,[18] 1998.

Table 1 continued.

Report	Place of practice	Procedure	Origin of mitochondria for transfer	Manipulation	Patient/s	Clinical consequences	Remarks
Kong et al.,[23] 2003	China	AGCMT	Autologous granular cells	Mitochondrial transfer	A 37-year-old woman with two miscarriages after IVF.	A triplet pregnancy. At the 5th week of pregnancy, one fetus ceased to develop. At the 30th week, two normal infants (boy and girl) were born via caesarian section.	First live births after AGCMT in mainland China.
Kong et al.,[24] 2003	China	AGCMT	Autologous granular cells	Mitochondrial transfer	A 46-year-old infertile woman.	A singleton pregnancy ended in a miscarriage at the 9th week.	About 3,000 mitochondria were transferred into each oocyte.
Zhang et al.,[12] 2003	China	PNT	Zygotes created using donor oocytes and spermatozoa of patient's partner.	Karyoplast transfer	A 30-year-old nulligravida woman who failed two IVF cycles.	A triplet pregnancy. After selective reduction of a fetus, the other fetuses prematurely delivered and died.	First reported at a conference in 2003. Peer-reviewed article published in 2016. Electrofusion of karyoplast and enucleated zygote was performed.
Fakih et al.,[26] 2015	Canada and the United Arab Emirates	AUGMENT	Autologous 'oogonial precursor cells'	Mitochondrial transfer	93 patients with a poor prognosis after IVF (aged 20–48 years old).	11 and 18-fold increase in ongoing clinical pregnancy rates.	The presence of 'oogonial precursor cells' is controversial.
Oktay et al.,[27] 2015	Turkey	AUGMENT	Autologous 'oogonial precursor cells'	Mitochondrial transfer	10 women with >2 IVF failures (aged 27–41 years old).	Clinical pregnancy attained in four of 10 recipients. One live birth, two miscarriages, one ongoing pregnancy at time of study publication.	The presence of 'oogonial precursor cells' is controversial.
Zhang et al.,[15] 2017	USA and Mexico	MST	Donor oocytes	Karyoplast transfer	A female with a mtDNA mutation (T8993G), multiple pregnancy losses and deaths of offspring due to Leigh syndrome.	A boy was born with a mtDNA mutation load of 2.36–9.23% in tested tissues. He was healthy at 7 months of age.	Electrofusion of karyoplast and enucleated oocyte was conducted in the USA, then, an euploid embryo was shipped to Mexico for transfer.

AGCMT: autologous granular cell mitochondrial transfer; AUGMENT: autologous germline mitochondrial energy transfer; ICSI: intracytoplasmic sperm injection; IVF: *in vitro* fertilisation; PNT: pronuclear transfer; OT: ooplasmic transfer.

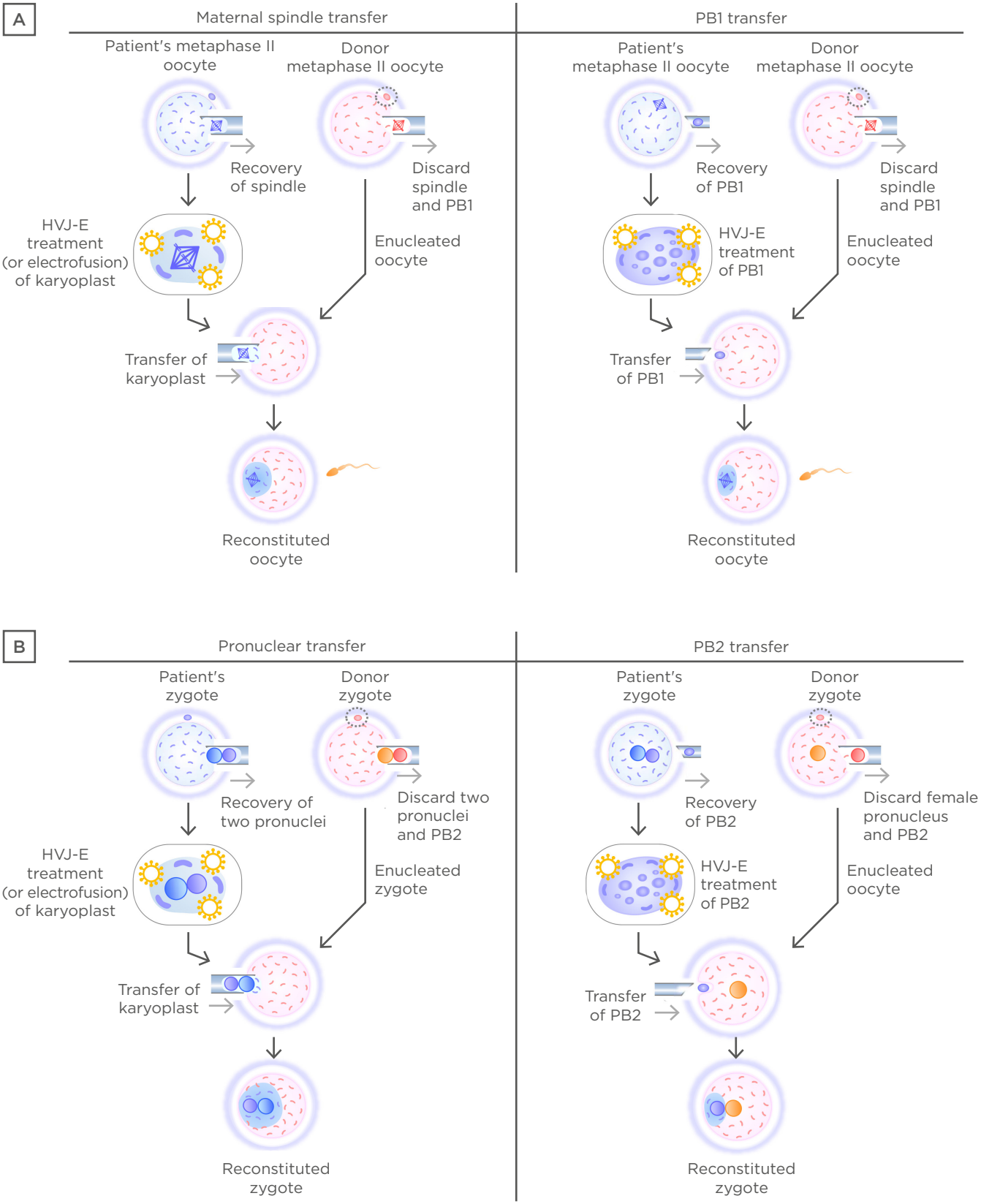

Figure 1: Procedures of maternal spindle transfer, first polar body transfer, pronuclear transfer, and second polar body transfer.

A) Procedures of maternal spindle transfer (left) and PB1 transfer (right). B: Procedures of pronuclear transfer (left) and PB2 transfer (right).

HVJ-E: haemagglutinating virus of Japan envelope; PB1: first polar body; PB2: second polar body.

Likewise, other OT cases intended as infertility treatment for women with a history of implantation failure and/or poor embryo development in women of ≥35 years of age can be found in Table 1. In typical OT, ooplasm from a fresh, mature oocyte donated from a fertile woman is transplanted into the oocytes of an infertile patient through intracytoplasmic sperm injection because electrofusion of the ooplasm and oocytes likely damages the viability of the resultant oocytes.[18] OT variants in the USA and Taiwan used frozen-thawed donor oocytes and donor tripronucleate zygotes as a source of ooplasm.[19,20] These efforts led to live births in some cases.[8,10,11,19-21] Aneuploidy, namely 45,X0 (Turner syndrome), was found in two different fetuses in the USA after OT, which resulted in a miscarriage and selective fetal reduction (Table 1). Furthermore, 1 of 17 children born via OT in the USA was diagnosed with a borderline pervasive developmental disorder (Table 1).[10]

Autologous Mitochondrial Transfer

Autologous granular cell mitochondrial transfer (AGCMT) does not depend on oocyte donation. In the three AGCMT cases from Taiwan and China, hundreds to thousands of mitochondria from the patient's own granular cells were injected into quality-compromised oocytes (Table 1).[22-24] Importantly, although AGCMT adds the patient's mitochondria to their own oocytes, it can potentially induce heteroplasmy in the injected oocytes by mixing mitochondria from somatic cells and germ cells in one individual.[25] AGCMT has led to live births as well as a fetal death and miscarriages. In 2015, two clinical reports from Canada, the United Arab Emirates, and Turkey reported the effects of autologous germline mitochondrial energy transfer (AUGMENT) on clinical pregnancy rates.[26,27] AUGMENT, which appears to be a derivative of AGCMT, uses mitochondria from the patient's oogonial precursor cells. However, the populations of the two studies included younger women of 20–27 years of age (Table 1). Furthermore, their study design, as well as the presence of oogonial precursor cells in older women, is controversial.[28-30]

Karyoplast Transfer

The first PNT implementation reported from China in 2003 intended to treat intractable infertility via karyoplast transfer using a larger micropipette[12] (30–40 µm, 5–6-times larger than the needle used in intracytoplasmic sperm injection) (Table 1, Figure 1B). The subject was a 30-year-old woman who experienced embryo arrest in infertility treatment; she had received two IVF cycles prior to PNT. PNT led to a triplet pregnancy; however, after selective fetal reduction, one of the fetuses died of respiratory distress and the other of cord prolapse. Despite a lack of detailed data, the report claimed that the karyotypes of the fetuses were normal, that the nDNA of the fetuses and the patient matched, that the mtDNA profiles of the fetuses and donor were identical, and that the patient's mtDNA was not detected in the fetuses. In PNT, electrofusion was performed to fuse the patient's karyoplast with an enucleated zygote, which differed from the technique in the USA OT study (Table 1).[18]

In 2017, a group led by the first author of the 2003 PNT report[12] published the first report on MST in a cross-border project between the USA and Mexico (Table 1, Figure 1A). MST differed from previous germline mtDNA modifications in that it used karyoplast transfer in oocytes to prevent the onset of mitochondrial disease (specifically Leigh syndrome) in offspring. The female subject had experienced miscarriages and the loss of offspring due to an ATPase gene mutation in her oocyte mtDNA. The mtDNA mutation load of the woman's oocytes was almost 100%. The mtDNA haplogroup of the patient and the oocyte donor were different (I and L2c, respectively). The heteroplasmy level in the blastocysts after MST was 5.7%, which was higher than the levels in other preclinical reports using human oocytes (undetectable or <1%).[31,32] This MST case led to the birth of a boy. However, the mtDNA mutation load of his tested tissues varied from 2.36–9.23%, and his long-term prognosis remains unclear because the reversal of a pathogenic mtDNA copy may happen.[33,34]

Other Procedures

In addition to PNT and MST, two types of karyoplast transfer have been proposed: germinal vesicle (GV) and aggregated chromosome transfer. GV transfer removes and transfers the nucleus surrounded by the

membrane in oocytes in the prophase of meiosis I.[35] Aggregated chromosome transfer is performed from the breakdown of the GV to the formation of the metaphase-I spindle, during which chromosomes are visible.[36] However, both procedures have not yet been used clinically.

More recently, newer germline mtDNA modification procedures have been proposed: first polar body transfer (PB1T) and second polar body transfer (PB2T).[37,38] In PB1T, a first polar body is transferred to an enucleated mature oocyte (Figure 1A). In PB2T, a second polar body is removed from a zygote and replaced with the female pronucleus in a donor zygote (Figure 1B). Polar body transfer may have advantages over MST and PNT in terms of mitochondrial carry-over because human polar bodies contain few mitochondria.[39] However, fusion of a polar body and karyoplast requires haemagglutinating virus of Japan-envelope treatment, the safety of which remains unknown in human reproduction. The histories of PB1T and PB2T are shorter than the histories of PNT and MST. Despite the successful production of mice using first or second polar bodies,[40] human reproduction involving polar body transfer is still a long way from clinical application; further research is required to ensure the safety of the resultant offspring.

The history of germline mtDNA modification began with the clinical use of OT in 1996. These initial techniques gave rise to variants, including autologous mitochondrial transfer in oocytes and karyoplast transfer in zygotes and oocytes. However, the characterisation of the mitochondrial functions and mtDNA profiles in patients and the resultant offspring was largely insufficient in such small-scale studies. Following the first MST procedure, the heteroplasmy levels of the patient and her baby were analysed; however, the rate of mtDNA carry-over was relatively high in the offspring. Low levels of heteroplasmy can lead to subsequent reversal of the original mitochondrial genotype in MST.[33,34] It is hypothesised that mtDNA haplotypes with specific D-loop polymorphisms are preferentially amplified, potentially causing the reversal.[34] Additionally, the need for matching between nDNA and mtDNA in MST and PNT is controversial. Some assert that mismatching between donor mtDNA and patient nDNA might cause dysfunctional respiratory chain,[41] while others disagree.[33,34,42] Thus, germline mtDNA modification that intervenes in evolutionarily-developed mitochondrial–nuclear interactions using micromanipulation remains largely experimental in human reproduction.

LEGALITY IN THE WESTERN WORLD

Although adverse events following OT and PNT for infertility treatment led to prohibition of germline mtDNA modification in the USA and China, the UK became the first nation to permit PNT and MST, for the sole purpose of preventing serious mitochondrial disease in offspring. In Europe, the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (ETS No. 164) was concluded in 1997 (the so-called Oviedo Convention).[16] This treaty, which is the only binding international law in the biomedical field, stipulates that "An intervention seeking to modify the human genome is only to be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants" (Article 13).[16] Since the Oviedo Convention appears to prohibit germline mtDNA modification for human reproduction, it is worth analysing the legality of germline mtDNA modification focussing on the Western world. Sixteen countries were selected based on observed activities, including clinical reports, trial registries, advertisements relevant to germline mtDNA modification.[13] Of the 16 countries, 10 ratified the Oviedo Convention; Germany, Italy, Northern Cyprus, Russian Federation, the UK, and Ukraine did not (Table 2).[16]

The domestic policies relevant to germline mtDNA modification in the 16 countries were further analysed (Table 2). France, Germany, and Italy legally prohibit mtDNA use in reproductive medicine. Conversely, Northern Cyprus, the Russian Federation, and Ukraine, are permissive to its use in reproductive medicine. In the remaining 10 countries, the UK maintains the legal prohibition of all germline mtDNA modifications except PNT and MST for

disease prevention (use for infertility treatment is illegal). Southern Cyprus and Turkey only permit autologous mitochondrial transfer, such as AGCMT and AUGMENT. Domestic laws in the Czech Republic, Serbia, and Spain only prohibit PNT; the legality of other procedures is ambiguous. The legality of germline mtDNA modification in Albania, Georgia, Greece, and Portugal is ambiguous because, despite their ratification of the Oviedo Convention, these countries appear to allow its use in reproductive medicine.

Thus, there is some ambiguity regarding the domestic legality of germline mtDNA modification in Southern Cyprus, Turkey, Czech Republic, Serbia, Spain, Albania, Georgia, Greece, and Portugal, which ratified the Oviedo Convention. The Oviedo Convention stipulated that "Each Party shall take in its internal law the necessary measures to give effect to the provisions of this Convention" (Article 1).[16] However, OT and AUGMENT are advertised on the internet and may be offered in those countries (Table 2). These findings suggest that these nine countries have delayed or neglected amending or enacting relevant regulations prohibiting germline mtDNA modification, as others suggest.[43] There are inherent legal issues surrounding Article 13 of the Oviedo Convention, which prohibits the introduction of "any modification in the genome of any descendants", considering the characteristics of germline mtDNA modification. For example, males who undergo germline mtDNA modification do not pass their mtDNA onto the next generation. In addition, there is no specific legal definition of the term genome.[16] Some may specifically interpret 'genome' to mean nuclear genome.[44] In contrast, 'nuclear DNA' and 'mitochondrial DNA' are used in the UK's regulations regarding mitochondrial donation. Additionally, some might narrowly interpret Article 13 as the prohibition of modifying a gene(s) in mitochondrial genome of oocytes or zygotes, although germline mtDNA modification changes the composition of the mitochondrial genome copies. Thus, it is suggested that the domestic policies in Western countries and the Oviedo Convention were never meant to regulate germline mtDNA modification.

ETHICAL ASPECTS

Although germline mtDNA modification is permitted or may not be unlawful in some countries, researchers in such countries are required to practice germline mtDNA modification with due consideration of its ethical implications.

Table 2: The policies regarding germline mitochondrial DNA modification in 16 countries.

Jurisdiction	Year of Oviedo Convention (1997) ratification	An interpretation of domestic policy	Relevant domestic legislation	Relevant provisions in legislation	Procedures indicated by a survey on relevant clinical activities*
Albania	2011	Ambiguous	Law 8876/2002 on Reproductive Health	Article 33	MST, PNT
Czech Republic	2001	Prohibitive of PNT. Ambiguous on other procedures	› Act on Research on Human Embryonic Stem Cells and Related Activities and on Amendment to Some Related Acts 227/2006 › Act on Specific Health Services 373/2011	Section 209b of Act 2006	OT
France	2011	Prohibitive	› Civil Code › Law 800/2004 on Bioethics (amended 2009, 2011)	Article 16-4 of Civil Code	OT
Georgia	2000	Ambiguous	Law on Health Protection 1997	Article 142	OT
Germany	Neither signed nor ratified	Prohibitive	Embryo Protection Law 1990 (amended 2001, 2011)	Section 5	OT
Greece	1999	Ambiguous	Law 3089/2002 on medically assisted human reproduction	Article 1455	OT
Italy	Signed but not ratified yet	Prohibitive	Law 40/2004 Rules in the Field of Medically Assisted Reproduction	Article 13	OT

Table 2 continued.

Jurisdiction	Year of Oviedo Convention (1997) ratification	An interpretation of domestic policy	Relevant domestic legislation	Relevant provisions in legislation	Procedures indicated by a survey on relevant clinical activities*
Northern Cyprus	Neither signed nor ratified	Permissive	› Law Regulating Human Cell, Tissue and Organ Transplantation Rules 57/2014 › Assisted Reproductive Treatment Centres and Assisted Reproductive Treatment Procedures Regulation 381/2016	None	OT
Portugal	2001	Ambiguous	Law on medically assisted procreation (32/2006)	Article 4, 9, 10	OT
Russian Federation	Neither signed nor ratified	Permissive	› Russian Federation Citizen's Health Protection Law (22.07.1993. Reg. No5487-I) › Order 67th of the RF Ministry for Health (Reg. No4452 24.04.03)	None	OT
Serbia	2011	Prohibitive of PNT. Ambiguous on other procedures.	No. 40/2017 and 113/2017 laws on biomedically assisted fertilisation	Article 49	OT
Southern Cyprus	2002	Permissive of autologous mitochondrial transfer. Prohibitive of other procedures.	Law 69 (I)/2015 on the application of Medically Assisted Reproduction	Article 18	OT
Spain	1999	Prohibitive of PNT. Ambiguous on other procedures.	› Law 14/2007 on Biomedical Research › Law 14/2006 on Assisted Human Reproduction Techniques	Article 33 of law 2007. Article 13 of law 2006.	OT, AUGMENT
Turkey	2011	Permissive of autologous mitochondrial transfer. Prohibitive to other procedures.	› Penal Code › Legislation Concerning Assisted Reproductive Treatment Practices and Centres 27513/2010 › Regulation on Assisted Reproduction Treatment and Assisted Reproduction Treatment Centres 29135/2014	Article 231 of penal code. Article 10 of legislation 2010.	AUGMENT
UK	Neither signed nor ratified	Permissive of PNT and MST for preventing serious mitochondrial disease in offspring. Prohibitive of other procedures.	› Human Fertilisation and Embryology Act 1990 (amended 2008) › Human Fertilisation and Embryology (Mitochondrial Donation) Regulations 2015	3, 26, Part 1 of Act 2008. Part 1 of Regulation 2015.	None
Ukraine	Signed but not ratified yet	Permissive	Ministry of Health Order No. 771, Instruction on Procedures for Assisted Reproductive Technologies 2008	None	PNT

*Sixteen countries were selected based on the survey regarding germline mtDNA modification-relevant reports, trial registries, and advertisements on clinic websites or medical tourism websites.[13]

AUGMENT: autologous germline mitochondrial energy transfer; OT: ooplasmic transfer; MST: maternal spindle transfer; mtDNA: mitochondrial DNA; PNT: pronuclear transfer.

Applicable Cases

The history of PGD suggests that germline mtDNA modification will initially be used for disease prevention rather than infertility treatment.[45] Moreover, mutations in any of the 13 protein-coding mtDNA genes have been linked with various forms of mitochondrial disease.[2] However, the link between genes in mtDNA and infertility is currently controversial.[3,4] Potential targets of germline mtDNA modification to prevent mitochondrial disease in offspring include women who have lost children due to mitochondrial disease and women with an inherited mutant gene in their oocyte mtDNA.[15,46] mtDNA modification use for such women is understandable as a safeguard against genetic disease in future children.[47,48] Although PGD may be used to avoid the birth of children with mitochondrial disease, the selection of embryos or oocytes is not applicable to women who only have oocytes

with a high mtDNA mutation load. In addition, PGD that simply selects for the embryo having the lowest heteroplasmy level is unlikely to eliminate the risk of transmitting mtDNA mutations.[49] Despite these limitations, in some countries the clinical rationale and assumed welfare of the offspring might justify the use of some germline mtDNA modifications for women with a pathogenic mtDNA mutation in their oocytes who want to protect the future of their children from serious mitochondrial disease.

Reproductive Options

Excluding autologous mitochondrial transfer, the implementation of germline mtDNA modification requires oocyte donation. The direct use of donor oocytes can also help parents protect future children from life-threatening mitochondrial disease.[50] Donor oocyte availability suggests that the direct use of donor oocytes as well as germline mtDNA modification can be another reproductive option. Of course, many parents want to use PNT or MST to have a genetically-related child.[51] In contrast, some prospective mothers may be satisfied with the genetic relatedness between a resultant child and their partner. In the USA OT study, prospective parents considered the use of oocyte donation.[8] Thus, in addition to the experimental nature of germline mtDNA modification, the option of directly using donor oocytes should be explained to prospective parents.

Use of Preimplantation or Prenatal Testing

Prior to the transfer of embryos created via germline mtDNA modification, PGD can identify and exclude aneuploid embryos and embryos with an unacceptable level of heteroplasmy. Notably, PGD requires an additional intervention of cell biopsy, which can damage the viability of embryos.[45] This is particularly important when performing radical karyoplast transfer. Indeed, it was reported that physicians who plan to perform PNT in the UK were unwilling to use PGD.[52]

Instead, prenatal testing using amniotic fluid and chorionic villus sampling can confirm the genetic condition of a resultant fetus; however, invasive prenatal testing is associated with a miscarriage risk (approximately 1/300). Nevertheless, the use of prenatal testing should be carefully discussed because some parents would likely want to know whether germline mtDNA modification has been effective prior to the birth of their child. However, all treatments have risks. Prenatal testing may show that a pathogenic mtDNA mutation was not sufficiently reduced. In doing so, some women may feel distress over the decision of whether to maintain or terminate the pregnancy because they consented to experimental reproductive medicine to prevent their pathogenic mtDNA mutation from affecting their children. Due to the complicated ethics, prior sufficient counselling may be valuable for prospective women with a history of miscarriages or childbirths with mitochondrial disease.

Humane Follow-Up of Resultant Children

After the first MST, follow-up was initially planned until the resultant child reached 18 years of age.[15] However, the parents requested that no further genetic testing be undertaken, unless there was a clinical benefit for the child.[53] In 2016, Chen et al.[17] reported a survey result of 17 teenagers born from 13 couples that had used OT at a clinic in the USA between 1996 and 2001. Twelve of the 13 parents completed a questionnaire, while one parent did not respond to repeated requests. In addition, such parents did not agree to standardised clinical analysis due to a lack of disclosure to their children. Thus, the study ended in limited follow-up and possibly a high risk of bias.

It will likely be difficult to follow-up children born via germline genetic modification. However, when applying it to prevent the onset of mitochondrial disease in resultant children, the health of such children should be monitored. The period of follow-up is the most important question regarding the monitoring of such children.[54] The UK's policy on mitochondrial donation only requires physicians to prepare a follow-up plan for resultant children and parents need not consent to it.[55] Therefore, the author of this study argues that there is room for improvement in the UK's policy. Follow-up for several years, decades,

or even across generations may be necessary to confirm whether mitochondrial disease is successfully prevented and that no side effects develop. However, the lack of response from one parent in the OT survey[17] suggests that such long follow-up periods might infringe on privacy, dignity, and the welfare of the family. Thus, there may be a clash between clinical requirements and ethical considerations regarding the follow-up period of children born via germline mtDNA modification. Although this article cannot present a compelling solution, it is realistic and acceptable to perform follow-up for additional years after a primary endpoint (e.g., healthy birth) or until the resultant child becomes legally competent to refuse it.[56] Regarding the potential health risks in later life or transgenerational health risks, rigorous mouse experiments may provide meaningful evidence in advance because the generation time of mice is approximately 2 years.

CONCLUSION

The success of IVF in the UK has led to the worldwide spread of the technique since its first use in 1978. Likewise, if the first mitochondrial donation in the UK succeeds in preventing mitochondrial disease in a resultant child, the clinical use of PNT or MST will likely become legal, at least for disease prevention, in other countries. It is noteworthy that women who underwent mitochondrial donation experienced implantation failures and miscarriages.[45] Once PNT or MST for disease prevention is justified, it may be approved for treating intractable infertility in some countries. However, caution is required in its wider use from clinical, ethical, and evolutionary standpoints.

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Reproductive Function and Fertility in Women with Congenital Adrenal Hyperplasia

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Abstract

Congenital adrenal hyperplasia (CAH) refers to a group of disorders that are associated with defective adrenal steroidogenesis, the most common of which is 21-hydroxylase deficiency. The advent of neonatal screening, molecular genetics, and glucocorticoid and mineralocorticoid replacement has vastly improved the diagnosis and treatment of CAH; therefore, most infants and children with CAH successfully transition into adulthood. Several quality-of-life issues emanate from this transition, of which reproduction and fertility are notable. In this review, the authors appraise the effects of elevated androgens in CAH on the anatomic, hormonal, and psychosocial aspects of reproductive function. These CAH-associated alterations in reproductive anatomy or endocrine function can impair natural fertility, most often depending on the severity of CAH. In addition to assessing the fertility rates of women with CAH attempting natural conception, as well as those requiring assisted reproductive treatments, the authors also review data pertaining to the mode of delivery and pregnancy outcomes in these women. Finally, the importance of reproductive and preconception counselling in women with CAH attempting conception is briefly discussed.

INTRODUCTION

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder that is caused by defective steroidogenesis in the adrenal cortex.[1] Although frequently referred to as a single entity, CAH comprises different variants depending on the enzyme defect involved in adrenal steroidogenesis. Of all the CAH cases, >90% are caused by 21-hydroxylase deficiency.[2] These CAH cases can be further subdivided into classic CAH and the milder non-classic CAH.[3] The former comprises salt-wasting and simple-virilising forms of CAH. In the most severe salt-wasting form, deficiency of the 21-hydroxylase enzyme results in insufficient production of both aldosterone and cortisol. In contrast, the more moderate simple-virilising form of CAH is characterised by deficient cortisol but normal aldosterone production; thus, salt-wasting does not occur in this form. Both salt-wasting and simple-virilising CAH result in elevated androgens that cause virilisation of the external female genitalia. The non-classic form of CAH is associated with a mild 21-hydroxylase enzyme

defect. Consequently, this form of CAH results in only mild elevations of adrenal androgens that do not affect the external genitalia. It is important to note that non-classic CAH is more prevalent than classic CAH, i.e., 1 in 600 versus 1 in 16,000 cases, respectively.[4] In fact, the prevalence of non-classic CAH can be as high as 3.7% (4 in 100) in the Ashkenazi Jewish population.[5]

CAH may be also caused by other enzyme deficiencies, namely 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase/17-20 lyase, and cytochrome P450 oxidoreductase deficiency.[1] This review will focus on CAH caused by 21-hydroxylase deficiency.

EFFECTS OF CONGENITAL ADRENAL HYPERPLASIA ON REPRODUCTION AND FERTILITY

As adrenal steroid hormones are essential to normal sexual development and reproductive function, deficiencies of these hormones can impact a woman's reproductive fertility and function.[6] Thus, it is not surprising that CAH is the most common genetic disorder of steroidogenesis affecting fertility.[6,7] Several anatomic, hormonal, and psychosocial mechanisms have been proposed to explain the effects that CAH may have on normal reproductive function and fertility (Box 1). Many of the mechanisms listed below are not unique to 21-hydroxylase deficiency. Studies have highlighted that women with CAH due to other enzyme defects may have reproductive issues similar to women with 21-hydroxylase deficiency-induced CAH, though with varying presentations.[1]

Due to the virilising effects of androgens on the external genitalia, women with classic CAH have a smaller vaginal introitus, decreased vaginal lubrication, and decreased clitoral sensitivity, as well as significant dyspareunia.[8,9] These factors often contribute to a later sexual debut as well as ongoing anxiety about sexual performance.[8,10] This was exemplified by a cross-sectional study of 35 women with classic CAH; the study reported that 37% of women never had vaginal intercourse and that 81% of women experienced pain during intercourse.[11] The overall quality of sexual experience in women with CAH also tends to be lower than unaffected women, even after meticulous surgical genitoplasty.[12,13] Collectively, these factors result in decreased sexual activity, thereby diminishing the probability of natural conception.

Box 1: Summary of anatomic, hormonal, and psychosocial parameters affecting reproductive fertility and function in women with congenital adrenal hyperplasia.

Anatomic factors
› Smaller vaginal introitus, decreased vaginal lubrication, and decreased clitoral sensitivity in classic CAH.
› Higher rates of dyspareunia in classic CAH.
› Sexual debut at a later age and decreased sexual satisfaction in classic CAH, even if sexually active.
› Progesterone-mediated decrease in tubal motility and increase in thick cervical mucus.
Hormonal factors
› Elevated androgens are aromatised to oestrogens and suppress gonadotropin secretion.
› Elevated androgens may directly inhibit folliculogenesis.
› Preferential secretion of luteinising hormone that further increases androgen levels.
› Elevated progesterone levels in the follicular phase.
Psychosocial factors
› *In utero* exposure to elevated androgen levels in classic CAH can influence sex-role behaviour when reaching adulthood, potentially decreasing chances of pregnancy.
› Increased prevalence of non-heterosexual preference in classic CAH.

CAH: congenital adrenal hyperplasia.

CAH is known to alter the function of the hypothalamic–pituitary–ovarian axis. Potential aetiologies for the alterations to the hypothalamic–pituitary–ovarian axis include elevated androgens, elevated progesterone, expression of 5α-reductase in the ovary, or even a direct glucocorticoid effect.[8,14] Initial observations suggested that excess androgens are aromatised to oestrogen, which could suppress gonadotropin secretion.[15] Elevated androgen levels were also thought to inhibit folliculogenesis, albeit in rat models.[16] However, recent evidence has indicated that androgen excess impairs hypothalamic sensitivity to progesterone.[8] This causes increased gonadotropin-releasing hormone pulse frequency, resulting in a preferential secretion of luteinising hormone (LH). The hypersecretion of LH increases ovarian androgen production, which further potentiates and intensifies the effects of adrenal androgens.[8,17] Hypersecretion of LH is also noted in patients with polycystic ovarian syndrome (PCOS).[18,19] Therefore, there is considerable overlap between the manifestations of CAH and PCOS. In fact, patients with classic or non-classic CAH may often present with acne, hirsutism, alopecia, and oligomenorrhoea or amenorrhoea, which are pathognomonic of PCOS.[20-22] Furthermore, polycystic ovaries can be observed in approximately 40% of all women with non-classic CAH.[21] The association of chronic anovulation and irregular menstrual cycles with CAH can further decrease the fecundity of women with CAH.

Elevated progesterone levels can be detrimental to the reproductive potential of women with CAH. In contrast to the biphasic pattern of progesterone secretion in the follicular and luteal phases of unaffected women, progesterone levels are consistently elevated in women with CAH.[6,23] Elevated progesterone not only alters gonadotropin-releasing hormone pulse frequency but also decreases tubal motility, thickens cervical mucus, and diminishes endometrial receptivity.[24,25]

The issue of fertility is also closely associated with psychosexual development.[2] In addition to the effects on the external genitalia of women with classic CAH, *in utero* exposure to elevated androgens may influence sex-role behaviour.[26,27] For example, affected women are reported to exhibit increased male-type behaviour during childhood, as exemplified by toy preferences and aggressiveness.[26,28] While most women with CAH are heterosexual with a female sexual identity,[2] there is an increased prevalence of non-heterosexual preference in women with classic CAH, which correlates with disease severity.[29,30] Studies have also suggested that women with classic CAH may avoid heterosexual vaginal intercourse, especially in the presence of a small vaginal introitus,[31] thus contributing to apparent reduced fertility.

OVARIAN RESERVE IN WOMEN WITH CONGENITAL ADRENAL HYPERPLASIA

Ovarian reserve is a commonly used term to describe the reproductive potential in women, based on the quantity or reserve of remaining oocytes.[32] Different clinical markers, including antral follicle counts, ovarian volumes, serum levels of follicle-stimulating hormone, and anti-Müllerian hormone (AMH) on cycle Day 2, have been used in routine clinical practice to determine a woman's ovarian reserve. A recent prospective study compared ovarian volumes and AMH levels between 33 CAH patients and 33 age-matched controls.[33] There was a non-significant trend towards larger ovarian volumes in the CAH group (4.4 mL; range: 1.3–10.8 mL) when compared to controls (2.8 mL; range: 0.6–10.8 mL). In contrast, there was no significant difference in median serum AMH levels between CAH patients (11.0 pmol/L; range: 1–36 pmol/L) and controls (13.0 pmol/L; range: 1–45 pmol/L). Of note, median serum AMH levels were comparable in women with classic or non-classic CAH. Furthermore, there was no association between ovarian volumes, AMH levels, and androgen levels in CAH patients.

FERTILITY IN WOMEN WITH CONGENITAL ADRENAL HYPERPLASIA

Most data in the medical literature focus on the fertility of women with 21-hydroxylase deficiency; however, there have also been case reports and case series of fertility outcomes in women with CAH due to rarer mutations.[34-38]

Fertility in Women with Classic Congenital Adrenal Hyperplasia

The fertility potential of women with classic CAH generally mirrors the severity of their underlying disease.[6] Women with severe disease have significantly decreased sexual activity and overlap with PCOS-type anovulatory menstrual cycles, which cumulatively leads to decreased fertility rates.[39] It is important to note that women with classic CAH invariably require glucocorticoid and mineralocorticoid replacement to induce ovulatory menstrual cycles. Thus, natural conception in the absence of treatment is exceedingly rare. In fact, the earliest pregnancies in classic CAH patients coincided with the introduction of pharmacologic cortisol.[40]

Women with classic CAH can conceive while on routine maintenance therapy, and it is estimated that 80% and 60% of women with simple-virilising and salt-wasting forms of CAH, respectively, are fertile.[41] Most women who are compliant with maintenance therapy have ovulation rates as high as 40%.[42] However, some women require higher doses of glucocorticoids to adequately suppress adrenal androgens and initiate ovulatory menstrual cycles.[6] Despite these factors, women with classic CAH may have lower pregnancy rates when compared to unaffected age-matched controls.[43,44] In one Swedish study, 62 women with classic CAH were compared to 62 age-matched controls.[45] There was no difference in the age at menarche or first pregnancy between the groups. The frequency of irregular menstrual cycles and prior use of hormonal contraception was also comparable. However, the number of clinical pregnancies (31 versus 76) and live births (25 versus 54) was significantly lower among women with CAH. All children in the study had normal birth weight without any major malformations. Another study of 25 women with classic CAH in the UK reported similar pregnancy rates in CAH women (21/23, 91.3%) and age-matched controls in the normal population.[46] Yet, the fertility rate, defined as live birth rate per woman, was lower in the CAH group (0.25 per woman) when compared to the general population (1.8 per woman). An association has also been reported between *CYP21A2* mutation severity and fertility rates: no live births in the null group, 3% in the I2 splice group, and 33% in the Ile172Asn group.[45]

Fertility in Women with Non-Classic Congenital Adrenal Hyperplasia

While women with non-classic CAH are the least likely to be infertile, these women are more likely to be seen by medical providers due to the increased frequency of non-classic CAH over classic CAH and symptoms such as hirsutism or menstrual cycle irregularities.[6] In one study of 190 women with non-classic CAH, only 20 patients consulted for infertility.[47] Of the 190 women, 95 desired pregnancy, of which 85 conceived successfully. In these 85 women, 187 pregnancies occurred, resulting in the birth of 141 children in 82 of them. Overall, 57.2% of pregnancies occurred naturally without any treatment, 41.2% of pregnancies with hydrocortisone treatment, and 1.6% of pregnancies with ovulation induction agents. Of note, the rate of miscarriage was lower (6.5%) in women receiving glucocorticoid treatment compared to those without (26.3%). Of the 141 live born children, only 2 (1.5%) were born with classic CAH. A separate study of 203 pregnancies among 101 women with non-classic CAH reported a higher frequency of natural conception (68%) when compared to conception after glucocorticoid treatment (32%).[48] Similar to the results of the Bidet et al.[47] study, the spontaneous miscarriage rate was lower in the treated group (6.2% versus 25.4%). The rates of classic and non-classic CAH among the live born were 2.5% and 14.8%, respectively.

Fertility Treatments

Many women with classic or non-classic CAH may remain anovulatory despite adequate pharmacologic therapy or may have ovulatory cycles with elevated progesterone levels in the follicular phase.[49] In such cases, treatment with prednisolone 2–5 mg three times per day has been reported to decrease circulating progesterone levels during the follicular phase, thereby increasing the likelihood of natural conception.[1,46] Laparoscopic bilateral adrenalectomy, though controversial, can be used in select patients with elevated androgen and progesterone levels that are refractory to conventional medical therapy.[50] Despite its effectiveness, bilateral adrenalectomy can

increase the risk of future adrenal crises in patients with poor medication compliance.[51] Patients who still remain anovulatory or who are unable to conceive with the aforementioned treatments can benefit from ovulation induction with clomiphene citrate, aromatase inhibitors, or gonadotropin injections, and adjunctive metformin.[52-54] *In vitro* fertilisation (IVF) with or without preimplantation genetic diagnosis can be used in women if other fertility treatments are ineffective.[1,55]

As stated earlier, women with CAH may have symptoms that overlap with PCOS. Thus, anovulation and hyperinsulinaemia can be common in women with CAH. Prior studies have revealed that medications such as metformin or inositol, either alone or in combination, can mitigate insulin resistance and increase fecundity by increasing the frequency of spontaneous ovulation.[56] Furthermore, when used in IVF, metformin or inositol supplementation has been shown to increase oocyte and embryo quality.[57]

PREGNANCY OUTCOMES IN WOMEN WITH CONGENITAL ADRENAL HYPERPLASIA

Observational data have suggested that pregnancies in untreated women with classic or non-classic CAH are at a higher risk of spontaneous miscarriage.[45,47] However, these findings have not been confirmed in larger populations. Antepartum complications, such as pre-eclampsia and preterm birth, are similar in the CAH and general population. The mode of delivery in women with CAH, particularly those with classic CAH, remains a consistent concern. Studies have posited that early exposure to elevated levels of androgens may cause the maternal pelvis to assume a narrower, android configuration instead of the usual gynecoid pelvis.[58] Thus, while vaginal delivery is still possible,[59] the risk of labour arrest and subsequent caesarean delivery is high. Caesarean delivery may also be performed electively to prevent perineal trauma in women who have undergone surgical genitoplasty. In the Hagenfeldt et al.[45] study of 62 women with CAH, 25 women had children, of which 21 delivered via caesarean delivery, which was most often performed due to a history of prior genital surgery. In 2 patients, caesarean delivery was performed due to cephalo-pelvic disproportion, likely due to the inadequacy of the pelvis in allowing fetal descent.[45] Current data also confirm reassuring neonatal outcomes in mothers with CAH. In fact, virilisation of female neonates is rare even in mothers with classic CAH, usually occurring due to poor medication compliance.[60,61] The lack of virilisation is attributed to the activity of placental aromatase (i.e., conversion of androgens to oestrogens protects the female fetus from virilisation).[61] The long-term physical and intellectual development of children born to CAH mothers has also been normal.[44]

PRECONCEPTION COUNSELLING

Careful preconception counselling should be undertaken in all women with CAH contemplating pregnancy.[49] This counselling should include the risk of CAH transmission based on carrier status and CAH genetics, as well as possible pregnancy complications. Most studies suggest that the carrier frequency for classic CAH is approximately 1 in 62; the carrier frequency for non-classic CAH ranges between 1 in 5 and 1 in 16 depending on the population assessed.[62,63] It is particularly important to screen and counsel women with non-classic CAH for severe mutations that are phenotypically silent.[49] Data from the Moran et al.[48] study of 101 women with non-classic CAH reported a risk of 2.5% and 14.8% of conceiving a child with classic and non-classic CAH, respectively.[48] Some investigators have proposed that centres offering assisted reproductive treatment should consider screening infertile patients for CAH given the overlap between CAH, infertility, and PCOS.[62] In some cases, infants with classic CAH have been born after IVF to women harbouring CAH mutations that remained undiagnosed prior to treatment.[62]

Prenatal treatment of CAH also deserves special mention. Administration of dexamethasone before the 9th week of gestation can suppress adrenal androgen production and prevent virilisation of the external female genitalia. However, prenatal treatment of CAH remains controversial. This is primarily due to the potential impact of glucocorticoids on the

developing fetal brain and postnatal growth and development.[64]

CONCLUSION

As highlighted in the current review, CAH can have a profound impact on the synthesis of various adrenal steroids that may result in various reproductive issues. The detrimental effects of CAH on natural conception may occur due to alterations in reproductive anatomy or endocrine function. While patients with CAH have normal ovarian reserve parameters, they frequently require pharmacologic maintenance therapy to induce ovulatory menstrual cycles when attempting conception. Suppression of androgens from the adrenal gland is usually the first therapeutic strategy to achieve spontaneous ovulation and conception. Ovulation induction agents or IVF can be used in CAH patients who are unable to conceive naturally or with anovulatory menstrual cycles despite adequate treatment. To avoid perineal trauma, elective caesarean delivery is performed in women with a history of genitoplasty. Current data indicate reassuring pregnancy outcomes for CAH mothers, as well as long-term developmental and health outcomes of children born to CAH mothers. Finally, careful preconception counselling should be undertaken in CAH patients contemplating pregnancy.

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Active Selection and Single Embryo Transfer: Insights from Virtual Trials

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Abstract

Transferring embryos that are most likely to successfully implant and develop is important in optimising the efficiency of assisted conception. Slow-freezing of spare embryos has a high attrition rate; thus, actively selecting a viable embryo for a fresh transfer can theoretically result in a superior cumulative live birth rate compared to a conventional assessment of morphology. However, with vitrification and its much lower attrition rate, active selection may not deliver an improved cumulative live birth rate, as more viable embryos may be excluded due to the limitations of the technique than are lost due to warming attrition. For some women, the principal benefits of active selection techniques are likely to be associated with a reduction in the number of miscarriages and a reduced time to achieve a successful pregnancy or start another stimulated cycle. Active selection procedures need to be safe, accurate, and effective, without jeopardising the chance of a live birth. The analysis presented in this paper shows that, from the perspective of a self-funding woman, adding a costly active selection option is entering into a lottery for a better result that is most likely to offer no advantage and even the possibility of an inferior outcome for some. Gauging willingness-to-pay to avoid miscarriage and to reduce treatment time is likely to be complex, and depends on who is making the decision and how they are counselled. Evaluating cost-effectiveness, for which the unit of health is one live birth, is unlikely to be helpful in supporting a case for public funding or private insurance for a better selection technique. The author of this paper explores the theoretical potential of active embryo selection to optimise a full cycle of assisted conception, with particular reference to single embryo transfer.

INTRODUCTION

Effective embryo selection and successful cryopreservation of spare embryos are important to optimise the efficiency of a stimulated cycle of assisted conception.[1] The cryopreserved embryo survival rate has improved with the use of the newer vitrification technique over slow-freezing,[2,3] and the process now has clinical outcomes comparable to fresh transfer for ovulatory women.[4] Successful selection of viable embryos offers the potential to achieve a healthy singleton live birth event following the fewest possible number

of transfer procedures and to reduce the risk of miscarriage. It is now possible to transfer embryos one at a time without jeopardising the chance of pregnancy and avoiding clinical complications associated with multiple gestations.[1,3,5]

Abnormal embryo morphology is often associated with genetic abnormalities, and culturing embryos for as long as possible allows many unsuitable embryos to arrest naturally.[6] Active selection of surviving embryos may involve morphological, developmental, and genetic criteria that could have some value in differentiating viable and non-viable embryos prior to transfer.[7,8] Advances in technology offer increasingly more effective and objective assessment of embryo viability. Hot topics include time-lapse systems for embryo imaging[9,10] and preimplantation genetic testing for chromosome aneuploidy (PGT-A) using array comparative genomic hybridisation or next-generation sequencing;[11,12] however, what constitutes appropriate validation and implementation into routine practice is the subject of much debate,[13-15] and implementation and uptake of these technologies vary worldwide.

The aim of this article is not to evaluate the numerous technologies and approaches to assessing embryo viability at different development stages, but to explore the theoretical potential of active embryo selection to optimise a full cycle of assisted conception, with particular reference to single embryo transfer.

EFFECTIVENESS OF ACTIVE EMBRYO SELECTION IS SENSITIVE TO PREDICTIVE VALUE AND CRYOPRESERVATION EFFICIENCY

Using a previously published model,[16] Figure 1 shows the hypothetical effect of cryopreserved embryo survival on the cumulative live birth rate (CLBR) for women with two blastocysts suitable for transfer compared to a more effective selection method (test) with conventional morphological assessment. From the test perspective, the negative predictive value (NPV) is the proportion of normal (negative) test results that correctly predict a live birth, and the positive predictive value (PPV) is the proportion of abnormal (positive) test results that correctly predict no live birth.

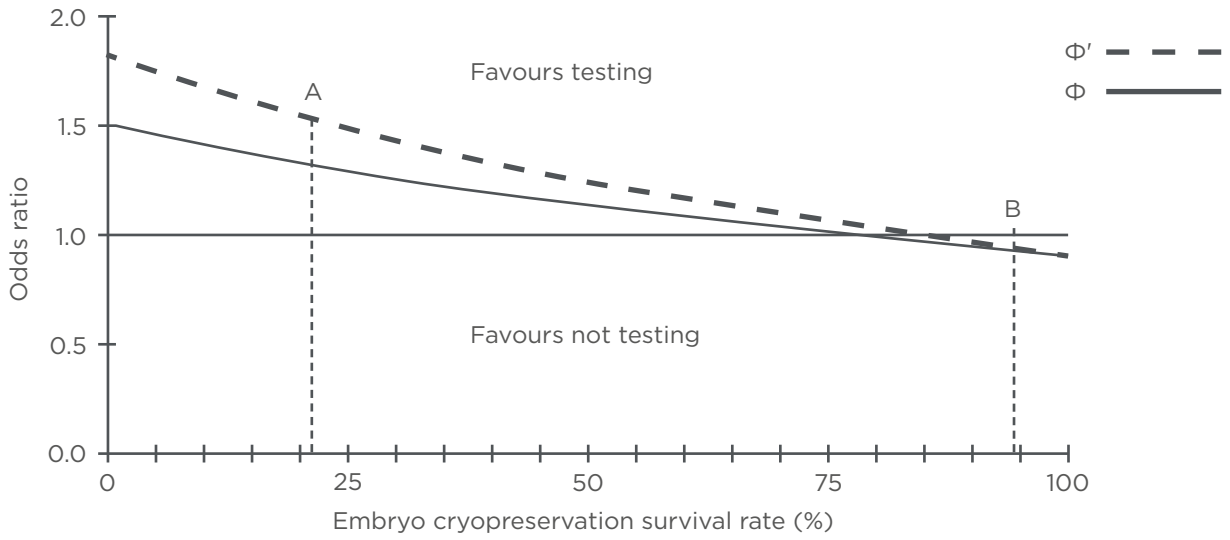

Figure 1: The hypothetical effectiveness of active selection versus a conventional embryo morphology assessment and cryopreservation survival on the cumulative live birth rate for women with two embryos suitable for transfer or testing.

Blastocyst survival rates: 21.4% (A) and 94.5% (B).

Φ: NPV 41.4%, PPV 96.0%; Φ′: NPV 62.5%, PPV 97.2%; NPV: negative predictive value; PPV: positive predictive value.

Adapted with permission from Scriven.[16]

It is postulated that embryo selection is more effective than morphological assessment alone when using PGT-A for every chromosome with a genetic microarray (Φ: NPV 41.4%, PPV 96.0%),[16] and would be even more effective with a better test (Φ′: NPV 62.5%, PPV 97.2%).[16] The incidence of viable embryos is estimated to be 25.4%, and the clinical pregnancy miscarriage rate to be 8.5% without genetic testing and 5.1% following genetic testing. Putative non-viable embryos with an aneuploid test result are excluded from a fresh or subsequent warmed transfer.

Using intact blastocyst survival rates of 21.4% (A) and 94.5% (B) for slow-freezing and vitrification, respectively,[17] and compared to a standard morphological embryo assessment, per 100,000 women it is estimated that:

- PGT-A Φ with slow-freezing: 6,040 (35,529 versus 29,489) more women could have a live birth, with 828 (1,903 versus 2,731) fewer clinical miscarriages and 30,042 (85,916 versus 115,958) fewer single embryo transfer procedures.
- PGT-A Φ′ with slow-freezing: 9,654 (39,143 versus 29,489) more women could have a live birth, with 634 (2,097 versus 2,731) fewer clinical miscarriages and 53,329 (62,629 versus 115,958) fewer transfers.
- PGT-A Φ with vitrification: 1,997 (41,355 versus 43,352) fewer women could have a live birth, with 1,798 (2,216 versus 4,014) fewer clinical miscarriages and 70,463 (100,005 versus 170,468) fewer embryo transfer procedures.
- PGT-A Φ′ with vitrification: 1,744 (41,608 versus 43,352) fewer women could have a live birth, with 1,785 (2,229 versus 4,014) fewer miscarriages and 103,895 (66,573 versus 170,468) fewer transfers.

A sufficiently accurate test increases the likelihood of selecting a viable embryo for a fresh transfer, avoiding the relatively high attrition associated with slow-freezing, resulting in a superior CLBR compared to conventional assessment. However, a test that is substantially better at selecting viable embryos for transfer can be inferior for live birth when combined with vitrification because relatively more viable embryos are excluded due to incorrect, abnormal (false-positive), or inconclusive results than are lost due to warming attrition. A warming survival rate of 94.5% may be considered modest; however, Figure 1 shows that higher rates have a negligible effect on the odds ratio (OR).

Since the unit of health is one live birth, adding an active selection test, which results in fewer live births than using conventional morphological assessment, cannot be cost-effective.[16] However, there are likely to be fewer clinical miscarriages because most aneuploid embryos will be excluded from transfer, and fewer transfers are likely to reduce the time required to complete a full cycle for some women; gauging the willingness-to-pay is likely to be complex and depends on who is making the decision.

INSIGHTS FROM A HYPOTHETICAL CLINICAL TRIAL

In the UK, the current National Institute for Health and Care Excellence (NICE) guidelines covering diagnosing and treating fertility problems in the UK recommend a single embryo transfer (fresh or cryopreserved) in the first full *in vitro* fertilisation (IVF) cycle for women aged <37 years, and more if there are ≥1 top-quality embryos for women aged 37–39 years.[18] In theory, although not always in practice, state funding is available for IVF, excluding the addition of PGT-A.

Figure 2 and Table 1 show the hypothetical outcomes from a virtual trial[19] conducted to provide insight into the cost-effectiveness of incorporating PGT-A into a first treatment attempt for every woman <40 years old (median age: 33 years; range: 22–39 years) to achieve a first live birth delivery. Fresh and vitrified-warmed embryos (if available) were transferred one at a time in a first complete full cycle (no dropout), comparing selecting out embryos (exclusion from transfer of putative aneuploid embryo) and ranking-only (transferring putative euploid embryos first and no exclusion) with conventional morphological assessment without additional testing. It is assumed that the vitrified-warmed embryo survival rate is 94%, and the NPV and PPV of the genetic test are 40.4% and 95.2%, respectively, with an incidence of 29.4% viable transferable embryos.

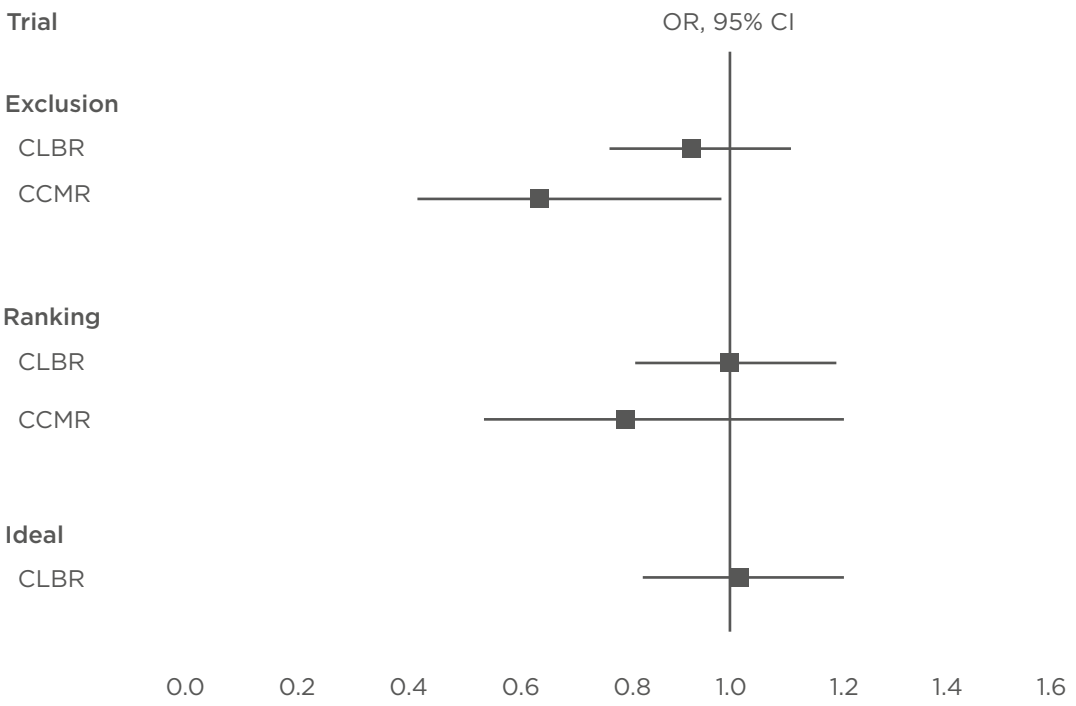

Figure 2: Results from a virtual trial and the hypothetical effect of incorporating preimplantation genetic testing for chromosome aneuploidy into a first treatment attempt for every woman <40 years old and the active selection on the intention-to-treat cumulative live birth and clinical miscarriage rates when transferring single embryos at a time.

CLBR: OR >1 favours testing; CCMR: OR <1 favours testing.

CI: confidence interval; CLBR: cumulative live birth rate; CCMR: cumulative clinical miscarriage rate; OR: odds ratio.

Adapted with permission from Scriven.[19]

With only conventional assessment, the live birth rate per transfer was 28.0% and the clinical pregnancy miscarriage rate was 10.0%; it took up to 43 months to complete a full cycle with up to 10 transfer procedures. Maternal age is an important independent predictor of live birth and in the study a younger woman (<38 years old) had a 2.6-times (p<0.0001) higher chance of a live birth than an older woman (38–39 years old). Costs are based on UK pound sterling 2017 prices:[19] IVF cycle (£3,300), intracytoplasmic sperm injection (£900), stimulation drugs (£900), embryo cryopreservation and storage (£800), warmed embryo transfer (£1,400), drugs (£150), and PGT-A (£2,950). The study was powered to detect a 40% reduction (3.6% versus 6.0%) in the cumulative clinical miscarriage rate (CCMR) (single-sided, 80% power, alpha 5%). An ideal scenario is also considered where only a viable embryo (if available) is transferred first with no risk of miscarriage, which has the optimum CLBR of 65.2%.

Considering only the first transfer attempt odds, a woman had a 1.699-times (39.8% versus 28.0%; p<0.0001) and 1.614-times (38.6% versus 28.0%; p<0.0001) higher chance of a live birth using exclusion and ranking-only, respectively, indicating that PGT-A is more effective than conventional morphological assessment to select viable embryos. Women who achieved a clinical pregnancy had a 0.728-times (7.5% versus 10.0%; p=0.2643) and 0.726-times (7.4% versus 10.0%; p=0.2598) higher chance of clinical miscarriage with exclusion and ranking-only, respectively. Including cryopreserved embryos, the CLBR was similar with PGT-A (exclusion: 63.3%, ranking: 64.8%) and without genetic testing (64.8%); however, exclusion was more effective to avoid clinical miscarriage (CCMR: 3.7% versus 5.6%; OR: 0.648; p=0.0451) (Figure 2).

Data from the published virtual trial[19] are summarised in Table 1. The number of women with different permutations (A–I) of outcome are

presented, comparing PGT-A to a conventional morphological assessment, including measures for their superiority for a live birth and or clinical miscarriage, and time and cost. Two costs for genetic testing were used for each transfer strategy: the median 2017 price for PGT-A (£2,965) and the PGT-A cost required to make genetic testing no more expensive overall, including all 1,000 women intending to start treatment (£1,195 for exclusion and £598 for ranking-only).

For exclusion (£2,965 for genetic testing), 15 (1.5%) fewer women achieved a live birth due to rejection of viable embryos with a false-positive result (L and M). Nineteen (1.9%) women avoided clinical miscarriage (D and E), of whom 9 (0.9%) completed their cycle more quickly with reduced expense (D). Testing increased the time and expense for 7 (0.7%) women (J and K) due to the incorrect exclusion of viable embryos. A total of 130 (13.0%) women, had no embryos suitable for transfer or testing (H), which includes 15 cycles abandoned before oocyte retrieval. For 694 (69.4%) women, the cycle outcome was the same for live birth and clinical miscarriage but with greater expense (G and I); however, the cycle time was reduced for 245 women (G). For 135 (13.5%) women, the outcome for live birth and clinical miscarriage was the same, but in a shorter time period (median reduction: 6 months, range: 6–15 months) and less expensive (median reduction: £135, range: £135–£5,585) (F). Reducing the PGT-A cost to £1,195 marginally increased the number of women for whom testing was superior for miscarriage with less time and reduced expense from 9 (0.9%) to 18 (1.8%) (D). However, the number of women for whom the live birth and miscarriage outcome was the same but with reduced time (median reduction: 3 months, range: 3–15 months) and cost (median reduction: £355, range: £355–£7,355) was substantially increased from 135 (13.5%) to 380 (38.0%) (F).

Ranking-only was not inferior for live birth (L and M) but avoided fewer clinical miscarriages than exclusion (D and E) and reduced the time and expense for fewer women (D and F).

Table 1: Hypothetical outcome permutations (A-I) for 1,000 women from a virtual trial illustrating the cost-effectiveness of incorporating preimplantation genetic testing for chromosome aneuploidy into first treatment attempts for every women <40 years old to achieve a first live birth.

Outcome	Live birth	Miscarriage	Time	Cost	Exclusion (£2,965)	Exclusion (£1,195)	Ranking (£2,965)	Ranking (£598)	Ideal (£2,965)	Ideal (£2,496)
A	Superior	Same	Less	Less	0	0	0	0	1	1
B	Superior	Same	Same	Less	0	0	0	0	1	1
C	Superior	Same	More	Less	0	0	0	0	2	2
D	Same	Superior	Less	Less	9	18	5	10	38	38
E	Same	Superior	Less	More	10	1	5	0	16	16
F	Same	Same	Less	Less	135	380	72	243	320	320
G	Same	Same	Less	More	245	0	171	0	219	219
H	Same	Same	Same	Same	130	130	130	130	130	130
I	Same	Same	Same	More	449	449	596	596	273	273
J	Same	Same	More	More	6	6	20	20	0	0
K	Same	Inferior	More	More	1	1	1	1	0	0
L	Inferior	Same	Less	Less	1	7	0	0	0	0
M	Inferior	Same	Less	More	14	8	0	0	0	0

Adapted with permission from Scriven.[19]

The number of women for whom the cycle cost was more expensive with genetic testing with the same outcome was increased from 449 (44.9%) with exclusion to 596 (59.6%) with ranking only (I).

The ideal test produced a superior live birth outcome for four (0.4%) women due to a fresh transfer avoiding cryopreservation attrition (A, B, and C); two of whom took more time due to a full-term gestation period. An optimal 54 (5.4%) women avoided a clinical miscarriage (D and E), of whom 38 (3.8%) were associated with less time and expense; however, assuming no overall difference in the total cost (£2,496 cost for genetic testing), 273 (27.3%) women had the same outcome with greater expense (I).

From the perspective of the self-funding individual, adding PGT-A is likely to be a costly lottery. Neither exclusion nor ranking-only of embryos should be expected to be superior for live birth, and the former strategy is likely to disadvantage some women. A very small minority of women are likely to benefit by avoiding a clinical miscarriage and, while a larger minority are likely to benefit by reducing the time to complete their cycle, most women are likely to pay more for the same outcome. However, some may have reduced costs associated with travel and accommodation if fewer visits to the assisted conception unit are required (not included in the analysis).

If it were possible to reduce the cost of a more effective active selection method, such that the overall total cost was the same as a conventional assessment, then a more persuasive case for public funding or private insurance might be made. Reducing the CLBR by excluding viable embryos incorrectly and transferring embryos with an abnormal (although possibly incorrect) result using ranking-only is likely to have some bearing on the willingness-to-pay. From the perspective of society, testing may also afford direct and indirect cost savings associated with managing fewer miscarriage and prenatal diagnosis procedures, and the upbringing of offspring with congenital disability (not included in the analysis). Potential savings associated with multiple pregnancy, preterm, and neonatal complications are unlikely to be significant since only one embryo is transferred at a time.[16]

CLINICAL TRIALS WITH CUMULATIVE OUTCOMES

Clinical prospective intention-to-treat embryo selection studies, including fresh and cryopreserved embryos, are likely to be costly, take years to complete, and have a risk of obsolescence. The European Society of Human Reproduction and Embryology (ESHRE) study into the evaluation of oocyte euploidy by microarray analysis (ESTEEM)[20,21] is a multicentre, randomised, double-blind trial with an intention-to-treat analysis of PGT-A with array comparative genomic hybridisation on polar bodies including women aged 36–41 years. The trial started in February 2012 and was completed in September 2017, but the final report has not yet been published. Initial results were presented at the ESHRE Annual Meeting in July 2017[22] and, following the randomising of 396 women, the study showed that the likelihood of a live birth within 1 year was not increased (41 versus 42 women with at least one delivery), but was achieved with fewer transfers using selection (178 versus 270).[23] The proof-of-principle study[20] that preceded the trial concluded that the ploidy of the zygote can be predicted with acceptable accuracy. Based on the pilot study, with 67.7% prevalence for aneuploidy the PPV (the proportion of abnormal test results that are aneuploid) and the NPV (the proportion of normal test results that are euploid) were estimated to be 94% (likely to be between 89% and 97%) and ˜100% (likely to be better than 93%), respectively,[24] with a substantial proportion (>10%) of normal zygotes likely to be excluded incorrectly.

Randomised controlled selection trials that attempt to estimate the CLBR are few. A recently published study,[25] which includes older women aged 38–42 years and excludes poor prognosis patients, reported a significantly higher live birth rate in the tested group following the first transfer: 52.9% (36 of 68) versus 24.2% (23 of 95) (OR: 3.522 [1.804–6.873]; p=0.0002), which is indicative of effective active selection of viable embryos. However, when adding live births from cryopreserved embryo transfers during the 6 months following the study recruitment period, the cumulative delivery rate in the tested group is similar: 37.0% (37 of 100) versus 33.3%

(35 of 105) (OR: 1.175 [0.662–2.085]; p=0.5285). It is not clear how many women who had not achieved a live birth during the study period still had cryopreserved embryos available. Fewer transfers were required to achieve a live birth and the time to pregnancy was reduced. It also seemed likely that around one in five women may avoid a miscarriage, although this may be an optimistic estimate.[19]

CONCLUSION

The advent of vitrification for embryo cryopreservation has changed the landscape of assisted reproduction. The principal benefits of active selection techniques are likely to be associated with a reduction in the number of miscarriages, and a reduced time to achieve a successful pregnancy or start another stimulated cycle. Active selection procedures need to be safe, accurate, and effective for these outcomes without jeopardising the chance of a live birth. Evaluating cost-effectiveness, where the unit of health is one live birth, is unlikely to be helpful in supporting a case for public funding or private insurance for a better active selection technique. Gauging willingness-to-pay to avoid miscarriage and reduce treatment time is likely to be complex and to depend on who is making the decision and how they are counselled. From the perspective of the self-funding individual, adding a costly active selection option is entering into a lottery for a better result, which may offer no advantage for most women and the potential for a worse outcome for some.

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