EMJ REPRODUCTIVE HEALTH

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+ ESHRE ANNUAL MEETING 2020

Reviewed

+ INTERVIEW

Prof Andrew Shennan, OBE spoke to us about his passion for global health equality

+ ABSTRACT REVIEWS

We are pleased to offer a range of engaging abstract summaries of research presented at ESHRE 2020

+ EDITOR'S PICK

When Regenerative Medicine Faces the Challenges of Reproductive Medicine: A Review Study on Recent Advances in the Strategies for Derivation of Gametes from Stem Cells

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Spencer Gore, CEO

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

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Front cover and contents photograph: Copenhagen, Denmark, home of ESHRE 2020. © atosan / 123rf.com

EMJ Repro Health 6.1

Welcome

Dear Readers,

It is again my pleasure to welcome you to this issue of *EMJ Reproductive Health*. This year, the Editorial Team at EMJ had the pleasure of attending the European Society of Human Reproduction and Embryology (ESHRE) virtual 36th Annual Meeting 2020. With 12,389 online attendees coming together to network and discuss the latest research, it is no surprise that this was a successful congress for ESHRE. We have compiled some of the most clinically topical research in our congress review, including the use of hair samples to assess fertility, results from the Endometrial Scratch Trial, and how oocyte yield can serve as a marker for age-related diseases.

Our fascinating abstract summaries from the congress were written by the authors themselves and provide you with the key findings of their work. These span multiple research areas including fragmentation of *in vitro* fertilisation, steroid metabolism in the eutopic endometrium, and polycystic ovary syndrome as an independent risk factor for gestational diabetes.

Our Editor's Pick this issue is the insightful paper by Juliá and Medrano, a comprehensive review of the derivation of germ cells and gametogenesis *in vitro* and in human and mice models. And we highly recommend that you peruse our thought-provoking Congress Session Review entitled "Stress and Infertility – The Chicken or the Egg," providing an account of the opposing views of Prof Boivins and Prof Lawson on the correlation between stress and infertility.

Within the journal is an interview with Andrew Shennan, professor of obstetrics at St Thomas' Hospital in London, UK. Prof Shennan shared insights into his passion for working with various charities, his association with the World Health Organization (WHO) and the International Federation of Gynaecology and Obstetrics (FIGO), as well as his appointment to the OBE.

Specialists in reproductive health will benefit from the wide range of content in our newest publication and we take great pride in presenting this to you. As always, I must thank the Editorial Board, authors, interviewees, and the readers for their ongoing support and collaboration that makes the journal a significant resource for all. We hope you enjoy reading this issue of *EMJ Reproductive Health* and we look forward to seeing you at the ESHRE Annual Meeting in 2021.

Spencer Gore

Chief Executive Officer, EMG-Health

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Foreword

Dear Colleagues,

It is my pleasure to welcome you to *EMJ Reproductive Health 6.1*.

The European Society of Human Reproduction and Embryology (ESHRE) 36th Annual Meeting, which should have been held in Copenhagen from July 5th to 8th 2020, was regularly held as a virtual meeting because of the COVID-19 pandemic. I had expected reduced participation in a virtual meeting; however, as usual, the meeting was very successful. All the sessions that I attended showed a very high number of participants from all over the globe, despite the different time zones. The virtual discussion was very active and proficient. The programme, as usual, considered all the main themes regarding natural and assisted reproduction, with particular attention to novelties.

This year, an interesting session was dedicated to political and juridical aspects of assisted reproductive techniques (ART). During this session, named 'Patient Priorities,' Debbie Kennett, an Honorary Research Associate in the Department of Genetics, Evolution, and Environment at University College London, London, UK, faced the important problem of gamete donors and recent legislation regarding the end of donor anonymity, whereas Anita Fincham, member of Fertility Europe, patients' advocate, and psychodietitian, reviewed what has been done and what should be done to eliminate all obstacles for free access to ART in European countries. Indeed, there are still too many countries in Europe where access to ART is limited to heterosexual couples, and/or with very restrictive legislations. Anita Fincham provided suggestions on what should be done to remove these obstacles.

As expected, there were many talks regarding the recent COVID-19 pandemic. Dr Gulam Bahadur considered vertical transmission of COVID-19 in a systematic review, concluding that vertical transmission is possible, with possible important consequences for the foetuses. All ESHRE 2020 lectures and sessions will be available online for subscribers until the end of the year.

This edition of *EMJ Reproductive Health* contains a compendium of interesting peer-reviewed articles encompassing several important topics related to reproductive health, which I am confident you will enjoy.

Kind regards,

Elisabetta Baldi
University of Florence, Italy

Available now.

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Feature

+ The COVID-19 Conundrum and Cancer – Making Perfect Sense of Imperfect Data

Interviews

+ Data from the AUGUSTUS Trial Adds an Important Piece to the Complex Puzzle of Antithrombotic Treatment for Those with Nonvalvular Atrial Fibrillation with Acute Coronary Syndrome and/or Percutaneous Coronary Intervention
+ Oral Prostacyclin Pathway Agents in Pulmonary Arterial Hypertension: An Expert Clinical Consensus

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+ Editor's Pick: Bone-Related Markers of Cardiovascular Disease
  Ernesto Maddaloni
+ National Institute for Health and Care Excellence (NICE) Guidelines on Cannabis-Based Medicinal Products: Clinical Practice Implications for Epilepsy Management Rhys H. Thomas
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Congress Review

Review of the European Society of Human Reproduction and Embryology (ESHRE) virtual 36th Annual Meeting 2020

Location: ESHRE virtual Annual Meeting 2020

Date: 5th–8th July 2020

Citation: EMJ Repro Health. 2020;6[1]:12-19. Congress Review.

DISTANCE and disease were no barrier to this year's annual meeting of the European Society of Human Reproduction and Embryology (ESHRE), their first to be held entirely online. The ESHRE virtual 36th Annual Meeting 2020 saw 12,389 clinicians, embryologists, and researchers come together online to share in the latest research, expert insights, and 'coffee room' discussions of reproductive and fertility medicine.

The canals of Copenhagen, Denmark, were a sorely-missed backdrop to the global meeting, and attendees missed the chance to stroll the picturesque harbourfront Nyhavn, take in the beauty of the home city of Hans Christian Andersen, or appreciate the wonder of the world's most environmentally friendly city. But participants of ESHRE 2020 built a new online community, celebrating their time together in virtual chat rooms, across social media, and by taking part in the annual ESHRE Fun Run from their own homes. In her opening welcome, Prof Christina Magli, Chair of ESHRE, highlighted the importance of these varied congress activities "to feel the sense of community that is so typical of ESHRE events."

Insights shared at the congress were an engaging split between clinical and basic science, but sessions highlighted the overlap of the two aspects of reproductive medicine and the focus on improving patient care. Participants shared in journal clubs, poster presentations, and virtual 'coffee room' discussions, alongside >260 oral presentations by expert speakers across 74 sessions.

Prof Magli opened the congress with a welcome address and outlined the timeline of decisions and actions undertaken by ESHRE to help address the ongoing COVID-19 pandemic. She highlighted the formation of the ESHRE COVID-19 Working Group, collating data on the impact of the disease on pregnancies and provision of care in reproductive medicine, and invited

"this is what ESHRE is, a big community where everybody wants to learn, to exchange experiences and to network in that spirit of sharing that is typical of us, the people of ESHRE"

all congress participants and ESHRE members to contribute to this global dataset. This cooperative spirit was embodied throughout the congress, where presentations demonstrated collaboration between countries and across disciplines in the name of scientific understanding and improved patient care.

Over 1,800 abstracts were submitted to ESHRE this year, showcasing the wide array of ongoing research in fertility and reproductive medicine. Amongst the hundreds of included studies was research analysing Nordic birth registries to determine whether cerebral palsy risk has changed over the past 20 years as a result of using *in vitro* fertilisation treatment, alongside a large-scale, randomised UK trial assessing the impact of endometrial scratch procedures on embryo implantation rates in *in vitro* fertilisation, as well as a summary of the first long-term study to share the success of fertility preservation techniques for helping women to conceive following fertility-affecting cancer treatments.

The virtual congress format provided many engaging presentations, with chat functions allowing audience interaction and the opportunity for participants to up-vote their preferred questions for presenters to address live. One fascinating, important session addressed the question of whether stress contributes to infertility, with >500 people attending to hear Prof Jacky Boivin, Cardiff University, Cardiff, UK, and Dr Angela Lawson, Northwestern University, Evanston, Illinois, USA, provide scientific evidence bases for the two competing arguments.

The impressive programme was honoured with the annual prizes awarded to standout presentations. The Basic Science Award for oral presentation was awarded to Dr Chih-Jen Lin, University of Edinburgh Centre for Reproductive Health, Edinburgh, UK, for his research on the histone variant H3.3 chaperone complex HIRA, while the Clinical Science Award for oral presentation was presented to Hugh Taylor, Yale School of Medicine, New Haven, Connecticut, USA, for his presentation of a Phase III trial of linzagolix on heavy menstrual bleeding caused by uterine fibroids. The work of Alexandra Claire Benoit, Antoine Béclère University Hospital, Clamart, France, was celebrated when her presentation, discussing a web-based patient decision aid of fertility preservation for women with breast cancer, received the Nurses Award for best oral presentation by a nurse.

The work of all clinicians, researchers, and scientists was celebrated at ESHRE 2020 through the Humans of ESHRE 2020 campaign. Providing insight into the experiences of 10 ESHRE members in the setting of the COVID-19 pandemic, this humanising initiative upheld the community atmosphere of the ESHRE event itself. In her Humans of ESHRE 2020 profile, Prof Magli commented on this spirit: "A connection through the cyberspace will not undermine our capacity of communication, because this is what ESHRE is, a big community where everybody wants to learn, to exchange experiences and to network in that spirit of sharing that is typical of us, the people of ESHRE."

ESHRE 2020 REVIEWED →

Cerebral Palsy Risk in Assisted Reproductive Technologies Has Fallen

FEWER children born following the use of assisted reproductive technologies (ART) have cerebral palsy, compared with 20 years ago. Reduction in twin birth rates and preterm birth rates are likely to have contributed to this >50% fall in cerebral palsy cases.

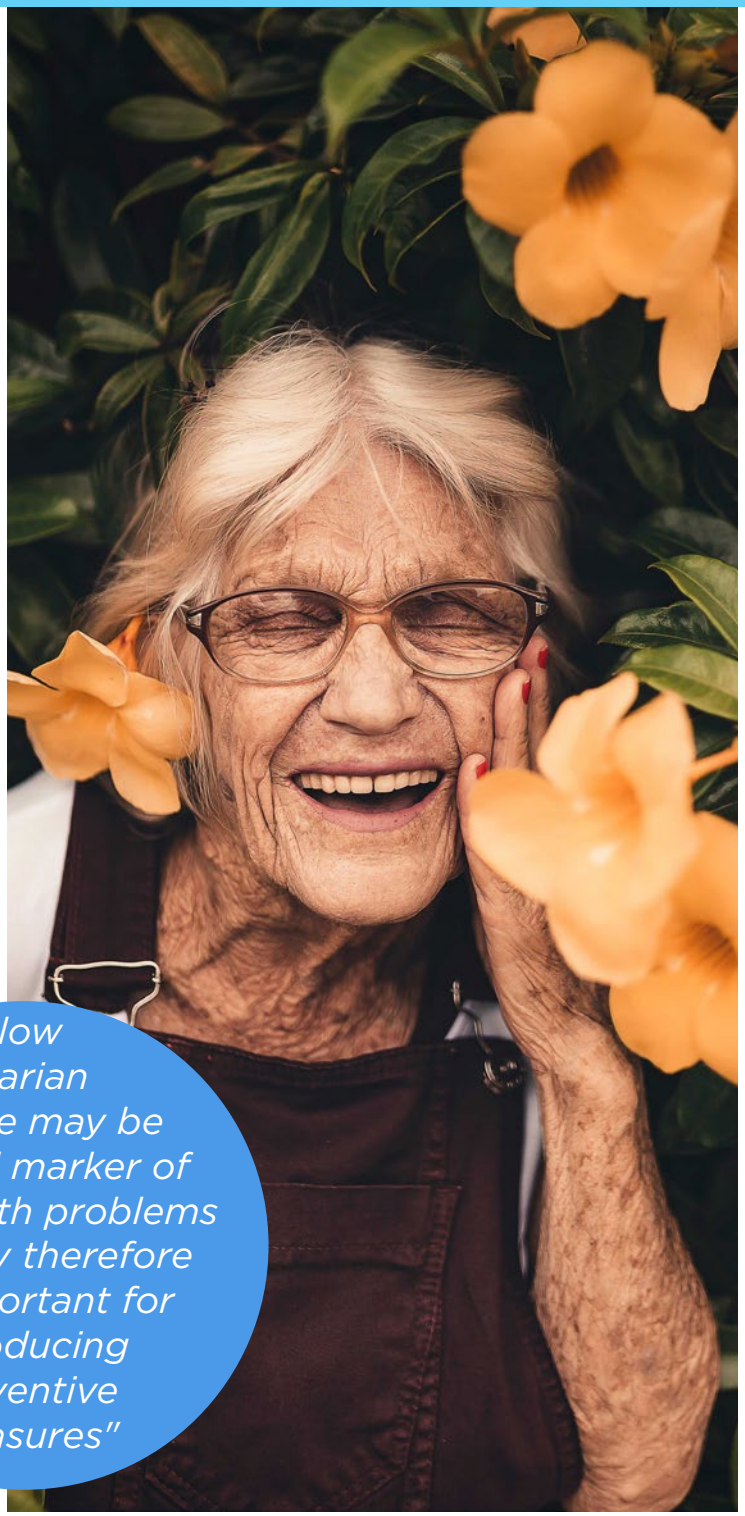
A Nordic study, presented at the ESHRE virtual 36th Annual Meeting, of 55,233 children born following ART, alongside 2,327,350 spontaneously conceived children, analysed the rates of cerebral palsy by year to determine the change over time. The registry-based cohort study used data from Denmark, Finland, and Sweden and adjusted for maternal age, parity, child's sex, plurality, country, and birth year.

Of the >55,000 ART-assisted births, 307 children were diagnosed with cerebral palsy (0.6%), compared to 5,911 spontaneously conceived children out of >2.3 million (0.3%) in the period 1990–2010. The data were studied within time periods (birth year 1990–1994, 1995–1999, 2000–2004, and 2005–2010). Comparing 1990–1994 to 2005–2010, rates of cerebral palsy decreased within the ART group (0.9% to 0.4%) and remained constant in the spontaneously conceived group (0.3%).

Further analysis considered the impact of singleton versus plural births on cerebral palsy rates for both groups. Risk of cerebral palsy was overall greater in ART singleton children compared with spontaneously conceived singletons (adjusted odds ratio [aOR] 1.32; 95% confidence interval [CI]: 1.10–1.57), but similar for twins between the two groups. Over the 20 years studied, there was a reduction in risk of cerebral palsy for the ART group versus the spontaneously conceived group (1990–1994 aOR: 2.88; 95% CI: 1.81–4.32; and 2005–2010 aOR: 1.34; 95% CI: 1.12–1.61).

"Limiting the number of twins born after ART treatment lowers the risk of CP in the ART population," explained Dr Anne Lærke Spangmose, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. She highlighted the importance of these findings in supporting a change in standard of care in ART from multiple to single embryo transfer: "Our findings emphasise that single embryo transfer should be encouraged worldwide."

Low Oocyte Yield in Assisted Reproductive Technology Associated with Increased Risk of Age-Related Diseases



OOCYTE harvest yield in assisted reproductive technology (ART), according to a new study presented in a press release at the ESHRE virtual 36th Annual Meeting dated 8th July 2020, can serve as a marker of accelerated ovarian ageing and increased risk of age-related diseases.

Early identification of women at risk of premature menopause has become increasingly important to initiate early preventive health initiatives. Repeated low oocyte harvest in ART is a marker of accelerated general ageing; however, could this also serve as a risk predictor of age-related morbidity and mortality? This was the hypothesis that drove the study of lead investigator and PhD student Mette Wulf Christensen, Aarhus University Hospital, Aarhus, Denmark.

"low ovarian reserve may be a useful marker of later health problems and may therefore be important for introducing preventive measures"

The study recruited the national registries of Denmark, dividing women ≤37 years old who had a first cycle of *in vitro* fertilisation or intracytoplasmic sperm injection between 1995 and 2014. The groups were based on their response to stimulation and consisted of: 1) those who produced ≥5 oocytes for collection, defined as 'early ovarian ages' (n=1,234); and 2) those who responded normally, producing ≥8 oocytes (n=18,614). The number of oocytes harvested in first and subsequent cycles was used as a marker of ovarian reserve and several national registers were applied to assess morbidity and mortality.

The 6-year average follow-up period showed that women in Group 1 had a 26% and 39% increased risk of all-cause mortality and cardiovascular diseases, respectively; increased risk of osteoporosis; and were more likely to be listed on the 'early retirement benefit' register compared to those in Group 2.

Although the risk of cancer and other age-related diseases was not statistically significant, Christensen noted that the data underlines an increased risk of age-related morbidity in young women with early ovarian ageing and she strongly supported the hypothesis that "low ovarian reserve may be a useful marker of later health problems and may therefore be important for introducing preventive measures such as lifestyle changes or the use of hormone replacement therapy to reduce the adverse health risks which follow an earlier menopause."

Hair Samples Could Be Used for Assessing Fertility

FERTILITY assessments are often conducted by testing blood samples for circulating biomarkers, including anti-Müllerian hormone (AMH). Researchers postulated whether this biomarker could be analysed in human hair, and the results were presented at the ESHRE virtual 36th Annual Meeting and in a press release dated 6th July.

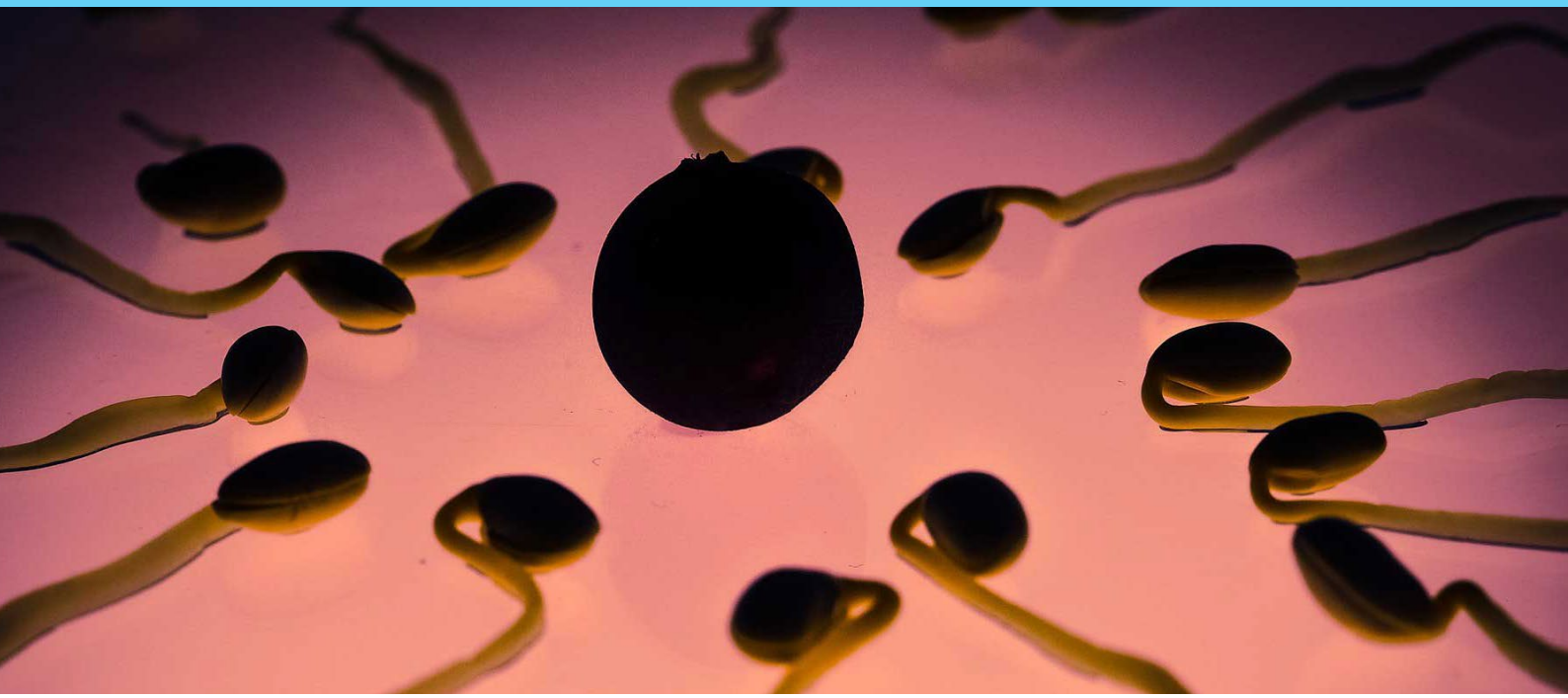
As a result of AMH being a product of granulosa cells of the preantral and small antral follicles in women, it is often used as a biomarker when assessing fertility. Obtaining blood samples invasively through median cubital vein punctures is the typical approach to test circulating AMH; however, hair samples may provide a superior understanding of the accrual of the hormone concentrations over longer periods of time. While steroid hormones have been analysed in hair for psychoneuroendocrinological studies, this is the first study to quantify AMH levels in humans.

In the prospective study, 152 females aged 18–65 years were included over a period of 10 months (recruitment is still ongoing). Blood and hair samples were collected in a clinical setting, but hair follicles were not required. An ultrasound to measure participants' antral follicle count was then performed. Once the biologically active AMH was extracted from the hair using a proprietary method, Western blotting was used to detect AMH presence in the hair extract. ELISA was used to measure AMH in plasma and serum.

A 70 kDa band representing AMH was successfully detected using Western blots in all samples (N=152). In the <25 years age group, AMH was detected in hair at an average level of 9.37 pg/mL (95% confidence interval [CI]: 6.77–12.00) and at 3.68 ng/mL (95% CI: 2.79–4.56) in serum. Detection results in the age group >39 years were much lower, with a mean of 3.02 pg/mL (95% CI: 2.19–3.85) in hair and 0.92 ng/mL (95% CI: 0.43–1.141) in serum samples. AMH measurements from hair correlated with age more strongly than plasma (p=1.26x10-5 [hair]; p=0.088 [serum]), and hair AMH levels also strongly correlated with antral follicle count.

The researchers commented: "We have a novel method of detecting AMH in a longitudinal matrix (hair) that could be a more appropriate representation of hormone levels compared to acute matrices like serum or saliva."

"We have a novel method of detecting AMH in a longitudinal matrix (hair) that could be a more appropriate representation of hormone levels"



Results from the UK Multicentre Endometrial Scratch Randomised Controlled Trial

ENDOMETRIAL scratch, a procedure in which a small biopsy is taken of the uterus lining, is performed in the hope of improving embryo implantation in first-time *in vitro* fertilisation (IVF) users. However, results from the UK Multicentre Endometrial Scratch Randomised Controlled Trial have revealed that this technique is no more effective than routine treatment.

These results were reported in a press release from the ESHRE virtual 36th Annual Meeting on 8th July 2020.

More than 1,000 females from 16 UK centres were enrolled in the study, in what is the largest trial of the procedure to date. The women were aged <37 years, were undergoing their first ever cycle of IVF, and were randomised equally to endometrial scratch or no scratch. The primary outcome was a live birth and the secondary outcomes included clinical pregnancy, implantation, ectopic pregnancy, miscarriage, preterm delivery, and stillbirth rates.

"Our study is the largest and most conclusive study in women having first-time IVF treatment, and the findings conclusively indicate that the practice of performing scratch in this group should stop"

The live birth rate of the intervention group was 38.6%, compared to 37.1% in the control group; there was no statistical significance. Additionally, there were no significant differences in secondary outcomes between the two groups: clinical pregnancy rate was 42.6% in the scratch group compared to 40.6% in the control group. Adverse event occurrence was similar between the two treatment arms and no deaths or neonatal deaths were reported.

Despite previous studies which have come to similar, discouraging conclusions, the endometrial scratch procedure is currently recommended to patients undergoing IVF in 83% of clinics in Australia, New Zealand, and the UK.

Dr Mostafa Metwally, chief investigator of the study, University of Sheffield, Sheffield, UK, stated that: "Our study is the largest and most conclusive study in women having first-time IVF treatment, and the findings conclusively indicate that the practice of performing scratch in this group should stop."

Positive Live Birth Rates for Fertility Preservation in Female Patients with Cancer: A 19-Year Study

FEMALES opting for fertility preservation (FP) at the time of cancer diagnosis may have good outcomes for utilisation and live birth rates. This is according to the results of a new study presented at the ESHRE virtual 36th Annual Meeting by researchers from the Assisted Conception Unit, Guy's and St Thomas' NHS Foundation Trust, London, UK.

Many countries offer the chance of FP with cancer care but there are limited data on the return of female patients to their stored gametes after cancer treatment, despite FP utilisation being on the rise. This prospective cohort study enrolled 879 females with a cancer diagnosis who requested FP counselling at Guy's and St Thomas' Hospital between January 2000 and December 2019. Data analysis was carried out using the patients' ages, anti-Müllerian hormone levels, and antral follicle count with a primary outcome of live birth rate and secondary outcome of return and utilisation rates (calculated by the number of patients who returned for follow-up and those who had undergone embryo transfer).

Follow-up assessment was attended by 297 patients (33.8%) for review of ovarian function, menopausal symptoms, hormone replacement therapy, and fertility treatment. Mean time taken to follow-up was 21.2±19 months, with 66.0% of patients returning for follow-up within 2 years after their cancer diagnosis. In total, 373 female patients received FP: 40.7% selected embryo cryopreservation, 53.4% selected oocyte cryopreservation, 5.1% had both treatments, and 0.76% underwent ovarian tissue cryopreservation elsewhere. The utilisation rate of females with stored gametes was 16.4% (61/373), the live birth rate was 72.1% (44/61), and the rate of miscarriage was 12.2% (8/61).

Notably, patients with breast cancer were more likely to return for follow-up of gamete utilisation compared to patients with other malignant diseases (44.3%), and they had higher live birth rates compared to patients with lymphoma.

Limitations included uncertainty of outcome for births from natural conception and underestimation of live birth rates because some patients require more time to attempt pregnancy. In the first publication on the utilisation rate after FP, the authors showed that one in six female patients who sought FP utilised gamete and/or embryo storage with a positive outcome.

Stress and Infertility – The Chicken or the Egg

Rachel Donnison
Editorial Assistant

Citation: EMJ Repro Health. 2020;6[1]:20-22.

FORTY-SIX percent of people think stress or emotional distress causes fertility problems, or the inability to achieve pregnancy with treatment. Stress, the response triggered from an imbalance between perceived threat and ability to cope with threat, was discussed in the session 'Stress and Infertility - The Chicken or the Egg' at the European Society of Human Reproduction and Embryology (ESHRE) virtual 36th Annual Meeting, which combined the presentation by Prof Jacky Boivin, Cardiff University, Cardiff, UK, who made the case for stress causing lack of pregnancy, followed by Prof Angela Lawson, Northwestern University's Feinberg School of Medicine, Chicago, Illinois, USA, who argued the opposing case.

"JUST RELAX AND IT WILL HAPPEN?" THE CASE FOR STRESS CAUSING LACK OF PREGNANCY

Stress manifests itself in many forms, explained Prof Boivin: there is the stress of the pregnancy waiting period, work-related stress, and relationship stress. Prof Boivin began her session by taking the audience through her research journey; she has spent most of her career arguing that there is no direct association between stress and infertility. She updated the audience on what is known so far: no organism is advantaged from never reproducing, and therefore animals have evolved to reproduce despite highly stressful environments. For instance, citizens living in areas with significant stressors such as poverty, political warfare, and scarce resources, can still maintain some of the highest fertility rates in the world.

Claiming that the stress-induced infertility direct models are too simplistic to capture stress effects on fertility, Prof Boivin advocated for the creation of more complex models, posing the question: what factors should be in the stress-fertility model, and what paths connect these factors to fertility?

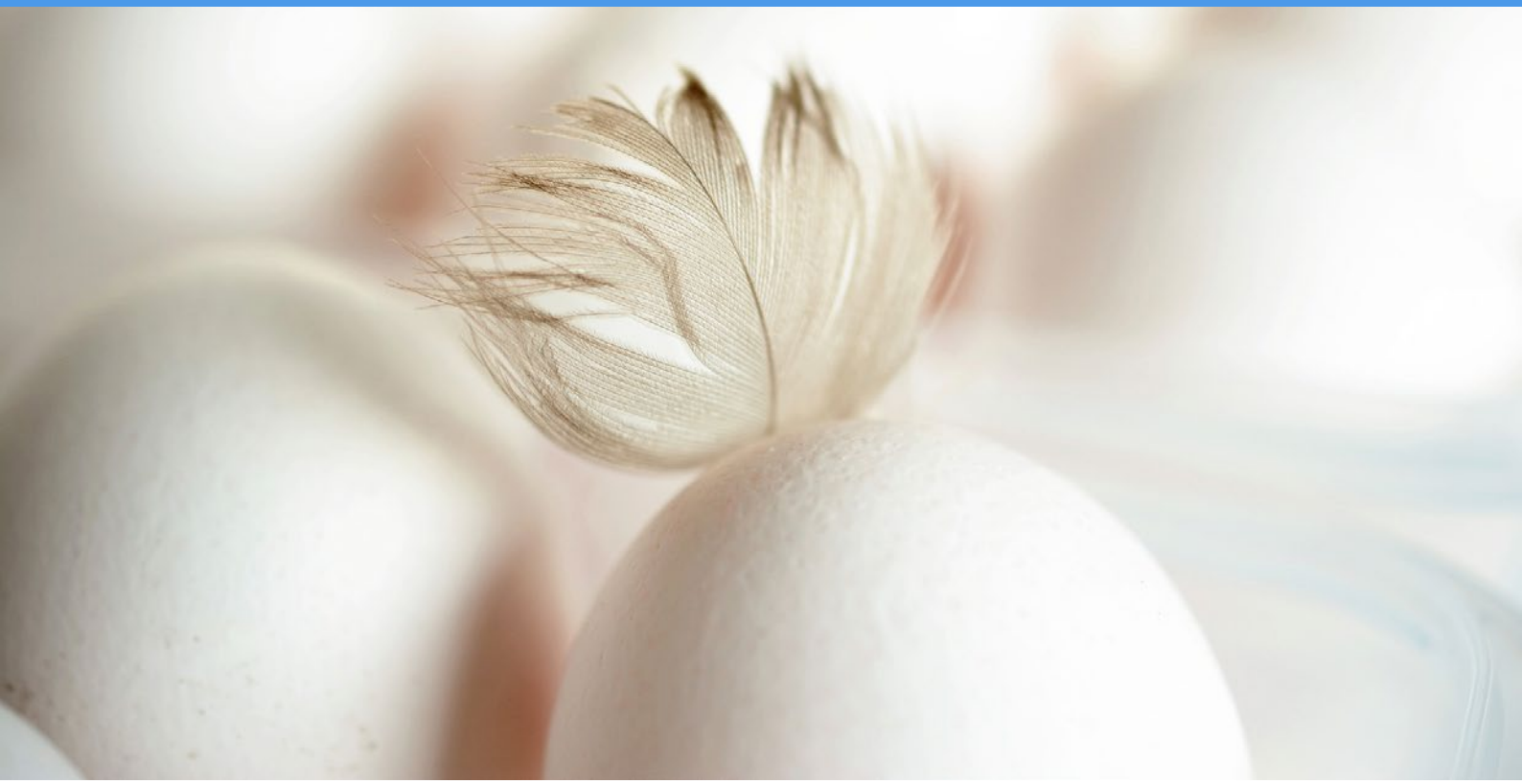
Stress-Induced Eating and Smoking

Taking into account the actions that people undertake when they are stressed is a good start, commented Prof Boivin. Stress-induced eating is one such coping strategy, which typically leads to a higher-fat diet. This can become a barrier to fertility, as obesity is associated with poorer pregnancy outcomes in both females and males.

Smoking is another factor that may be induced by stress, and studies have shown that smokers have a shorter latency to smoke under stress, and a higher number of puffs is consumed. Smoking has long been associated with poorer outcomes in assisted reproductive technology (ART); it has been linked to reduced semen quality, lower levels of ovarian stimulation, and fewer oocytes.

Behavioural Factors

Behavioural indices related to ART treatment continuation include depression at the start of treatment, meaning that patients are less likely to take up treatment or take longer to do so. Prof Boivin quoted a large Danish cohort study of almost 40,000 participants, which found that individuals with depression were less likely to take up treatment and less likely to achieve a live birth.



Stress-induced treatment drop-out is the final factor that Prof Boivin wished to include in the complex stress-infertility model. She relayed the results of a review which found that psychological distress in individuals made them five times more likely to discontinue treatment compared to people who had not experienced this. Prof Boivin was also careful to note that the depression could be a consequence of something else: "In fertility treatment, if couples are unsuccessful with treatment within the first 3 years… they are much more likely to have marital disruption," which can cause discontinuation of treatment and hence a lower chance of pregnancy.

Delayed decision-making is also a consequence of stress, which may be associated with reduced chances of pregnancy: "Comparing treatment when you're 39 to when you're 40, your chance of pregnancy drops substantially."

However, do we blame the patient? "No! Blame the poor access to affordable reproductive technologies and accumulated hardships and adversity that make trying to conceive so stressful."

Prof Boivin concluded that a new psycho-bio-behavioural model of fertility in ART should question: "At what point does stress start to induce infertility?" such as in ovarian and testicular function or fertilisation. An appreciation of individual stress contributions by both females and males should also be included, as well as the plausible direct and indirect paths between stress and infertility and the time component used to capture the treatment journey.

THE CASE AGAINST STRESS AS A CAUSE OF INFERTILITY

Consequence and Cause

Providing the counterargument for stress and infertility, Prof Lawson began with the statistic that one in eight individuals struggle to conceive, and that this struggle is associated with psychological distress. This distress starts "before fertility treatment even begins, can worsen across failed treatment cycles, and can become so severe as to equal that of newly diagnosed cancer patients."

Prof Lawson believed that this distress originates from the assumption that it is easy to conceive, and that often females and males are told that it is their fault. Self-blame then leads to loss of control, and individuals try to control their lifestyle habits instead. When this does not work, an individual's anxiety increases as they start to worry that they will not become a parent, and this results in symptoms of bereavement and grief as they lose a sense of their identity.

Biologic Plausibility

We have two competing systems, explained Prof Lawson: the sympathetic, or fight-or-flight, and the parasympathetic, or feed-and-breed,

nervous systems. The two systems vie for control of the hypothalamo-pituitary-gonadal (HPG) axis, which is the major neuroendocrine response system that controls the body's responses to stress. "There is a hypothetical shutdown of the HPG axis because of the release of stress hormones, cortisol and alpha-amylase, that may make it harder to get pregnant," continued Prof Lawson. However, research has shown that these systems never completely shut down as the body fights to maintain balance.

Choosing to back up her argument with meta-analyses instead of survey-based assessments, which she believed would be "cherry picking" of articles that are not representative of the entire literature, Prof Lawson conveyed the key take-home messages of several research studies on depression and anxiety, stress hormones, and relaxation.

Depression and Anxiety

The first article Prof Lawson evaluated showed no significant relationship between depression/anxiety and chances of conceiving. The second, however, did find a link between clinical pregnancy rates and anxiety or stress levels, though most of the significance was only through small statistical effect sizes. Finally, the third study did find significant differences between patients with depression/anxiety and infertility, compared to controls. Each of the meta-analyses found different results, which Prof Lawson explained is because the authors chose which survey-based assessments they would include, and which they would not; there were methodological problems such as small sample sizes, heterogeneity in outcomes, different measures of psychological distress, and, perhaps the biggest problem, the lack of appropriate control variables. "One particularly important control variable is knowledge of one's prognosis," stressed Prof Lawson, as well as a diagnosis of polycystic ovary syndrome or endometriosis, which are independently associated with psychological distress; no studies have controlled for these variables to date.

Stress Hormones and Infertility

The two primary stress hormones are alpha-amylase and cortisol. The research on cortisol is highly inconsistent, and the literature generally does not support a link to infertility. Conversely, the research on alpha-amylase has shown that in those who had higher levels of salivary alpha-amylase, there was a slightly longer time to pregnancy after 5 months of trying to conceive. Despite this, in one particular study, nearly 90% of the participants successfully conceived within the 12-month study period. These results confirmed what was already known about general populations and chances of conceiving: that many individuals will get pregnant within 6 months, and around 90% are successful within 12 months.

Prof Lawson suggested that this research has really been looking at changing hormones across menstrual cycles and the perimenopausal period. She stated that: "These changes in cortisol and alpha-amylase are associated with psychological distress, independent of changes in hormones."

Research on Relaxation and Pregnancy Chances

Additional meta-analysis data were then presented by Prof Lawson, this time on the topic of relaxation. Relaxing through psychotherapy was found to increase pregnancy chances, but only for patients who were not undergoing fertility treatment. Randomised controlled trials have failed to show a significant relationship between relaxation and likelihood of achieving pregnancy. Prof Lawson explained that the key take-away message here was that meta-analyses can be limited by the cherry picking of studies and can often include studies of poor quality; randomised controlled trials can be subject to selection and ascertainment bias.

Acupuncture is a relaxation method often talked about in terms of achieving pregnancy, but a 2013 Cochrane review showed no relationship between acupuncture and pregnancy chances. Yoga is another relaxation method, as well as music therapy, medical clowning, reflexology, and hypnosis; however, none of these have been tested in a randomised controlled trial.

In summary, Prof Lawson highlighted that overall, there has not been any evidence of a causal role in distress and infertility, unless one has functional hypothalamic amenorrhoea.

Despite their opposing views, Prof Boivin and Prof Lawson both agreed that the blame was never on the patient, and, in the words of Prof Lawson: "It is inappropriate to ever tell a patient to 'just relax.'"

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Abstract Reviews

Sharing insights from abstracts presented at the European Society of Human Reproduction and Embryology (ESHRE) virtual 36th Annual Meeting, global embryology and fertility researchers have provided these summaries of their fascinating studies.

Great Expectations: Patients Overestimate *In Vitro* Fertilisation Success

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Keywords: Assisted reproductive techniques, *in vitro* fertilisation (IVF), live birth, prognosis.

Citation: EMJ Repro Health. 2020;6[1]:24-25. Abstract Review No: AR1.

BACKGROUND AND AIMS

The general public is known to overestimate the success rate of *in vitro* fertilisation (IVF).[1,2] Qualitative interviews showed that well-informed females cryopreserving their oocytes were unrealistically optimistic about their chances for a live birth as they thought they and/or their gynaecologist would perform better than average.[3] To the best of the authors' knowledge, the live birth rates (LBR) expected by patients during their IVF cycle had yet to be studied and compared to the patient's personalised IVF prognosis. In addition, whether these expected LBR are affected by factors such as sex and dispositional optimism is unknown.

MATERIALS AND METHODS

The expected LBR and degree of dispositional optimism of consenting couples who had an oocyte aspiration in the Leuven University Fertility Clinic, Leuven, Belgium, between March and December 2019 were prospectively surveyed. Participants (male and female) were asked to each fill out their own questionnaire. Additionally, couples' personalised IVF prognoses were calculated using an adapted version of the van Loendersloot prognostic model after calibration on the authors' clinic's data (area under the receiver operating characteristics: 0.74).[4,5] The model predicts the chance of success of one 'complete' cycle (i.e., all fresh and frozen embryo transfers from the same episode of ovarian stimulation). Eligible couples completed at least one IVF cycle (second through to the sixth) with their own gametes after a previous IVF cycle with the same partner in the same clinic. The level of dispositional optimism was assessed with the reliable Revised Life Orientation Test (LOT-R) questionnaire.[6] The degree of misestimation was calculated with a formula: (expected IVF-LBR - prognosis)/prognosis. A positive sign shows overestimation, a negative sign shows underestimation, and the absolute value quantifies the extent of misestimation.

RESULTS

The 67 participating couples had a mean IVF prognosis (calculated LBR per completed IVF cycle, including fresh and frozen embryo transfers) of 31.8% (range: 4.8–59.4%; standard deviation [SD]: 16.90). Eighty-five percent of females overestimated their IVF-LBR (mean overestimation: 33.66%; SD: 20.02) and 47.8% expected their IVF-LBR to be more than double their calculated IVF prognosis (mean overestimation: 46.47%; SD: 16.10). Eighty-eight percent of males overestimated IVF-LBR (mean overestimation: 38.81; SD: 21.84) and 53.7% expected their IVF-LBR to be more than double of their calculated IVF prognosis (mean overestimation: 51.10%; SD: 17.75). Male patients expected significantly higher IVF-LBR compared to their female partners (64.4% versus 58.6%; paired t-test, p=0.028) and their degree of misestimation was also significantly higher (2.3 versus 1.8; paired t-test, p=0.013). Male and female partners did not differ in their levels of optimism (paired t-test, p=0.074) and the correlation between the level of optimism and expected IVF-LBR was rather weak (Pearson correlation coefficient in female patients: 0.428; p=0.000; and in male patients: 0.254; p=0.038). The correlation between the IVF prognosis and the level of optimism was also weak (Pearson correlation coefficient in female patients: 0.022; and in male patients: -0.163).

CONCLUSION

During IVF, patients, especially males, expected unrealistically high IVF-LBR and the difference between males and females was not explained by their level of dispositional optimism. Recruitment is ongoing to end up with a larger scale prospective cohort study with follow-up until the studied IVF cycle is completed by achieving a live birth or a negative pregnancy test after the transfer of the last (fresh and frozen) embryo. This study, however, already documented the extent of the interesting overestimation of IVF-LBR by couples going through IVF. Whether the couple's overestimation leads to distress in the case of a negative pregnancy test which could ultimately result in IVF discontinuation, as suggested by qualitative interviews, will be followed up.[7] In addition, couples' actual LBR and association with expected IVF-LBR and with prognoses will be assessed.

References

1. Lampic C et al. Fertility awareness, intentions concerning childbearing, and attitudes towards parenthood among female and male academics. Hum Reprod. 2006;21(2):558-64.
2. Skoog Svanberg A et al. Attitudes toward parenthood and awareness of fertility among postgraduate students in Sweden. Gender Medicine. 2006;3(3):187-95.
3. De Groot M et al. Perceptions of oocyte banking from women intending to circumvent age-related fertility decline. Acta Obstet Gynecol Scand. 2016;95(12):1396-401.
4. van Loendersloot L et al. Individualized decision-making in IVF: calculating the chances of pregnancy. Hum Reprod. 2013;28(11):2972-80.
5. Sarais V et al. Predicting the success of IVF: external validation of the van Loendersloot's model. Hum Reprod. 2016;31(6):1245-52.
6. Scheier M et al. Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the life orientation test. J Pers Soc Psychol. 1994;67(6):1063-78.
7. Peddie V et al. A qualitative study of women's decision-making at the end of IVF treatment. Hum Reprod. 2005;20(7):1944-51.

Evaluation of Fragmented Embryos: What Is the Best Way to Predict Its Implantation Potential?

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Keywords: Embryo fragmentations, embryo selection, time-lapse.

Citation: EMJ Repro Health. 2020;6[1]:26-27. Abstract Review No: AR2.

BACKGROUND AND AIMS

Fragmentation of *in vitro* fertilisation (IVF) embryos is used as a marker for embryo deselection.[1] Extensive fragmentation may be associated with reduced blastocyst formation and increased incidence of chromosomal abnormalities.[2] However, some of those embryos may still lead to pregnancy and delivery of a healthy child. Success rate prediction in these embryos is still an open question. Moreover, the cut-off of fragmentation rate that still enables achievement of pregnancy is not clearly defined.[3] It was hypothesised that using general and lab-specific models based on time-lapse technology may contribute to the selection of embryos with fragments.

MATERIALS AND METHODS

In this retrospective study, 4,210 embryos were analysed. They were incubated to the blastocyst stage in an EmbryoScope™ (Vitrolife, Gothenberg, Sweden) between 2013 and 2019 and included 379 embryos with >5% fragmentation. Embryos were selected for transfer or freezing versus deselection, based primarily on the general model for Day 5 development, provided by Vitrolife, and then re-examined by the laboratory's specific algorithms.

Participants, Materials, Setting, and Methods

Embryo fragmentations were measured using EmbryoScope tools by a senior embryologist. Percentage of fragmentation was documented twice for every embryo: at the first cell division and at their maximum volume. The patterns of fragment accumulation during embryo development were followed. Data were analysed using statistical methods for fragmentation with regards to patient age, insemination method, blastocyst formation, embryo transfer or freezing, known implantation data of the embryo, clinical pregnancy, and live birth rate.

RESULTS

The specific model score and a fragmentation percentage of up to 32% were found to be independent variables. Embryos with up to 20±12% fragmentation still had a high score according to the first division time and were usually transferred or cryopreserved. Significant differences in the fragmentation rate were found between embryos which reached the blastocyst stage and embryos which failed to develop (15±11% and 36±20%, respectively; $p<0.0001$).

Fragmentation usually appeared at the first division and this worsened during the third or fourth divisions. While no difference was found in fragmentation between embryos of standard IVF or intracytoplasmic sperm injection, age had a significant negative effect on fragmentation ($p<0.0001$). In this population of fragmented embryos, 33 out of 379 embryos resulted in the delivery of a healthy child, 104 failed to develop, and information was not available for the remaining 242, mainly because they were frozen without thawing.

In 92.8% of patients, more than one embryo had fragments. In 64% of patients, fragmented embryos were found in more than one cycle. All positive known implantation data had a maximum of 30% fragmentation, except for one embryo with 43% fragmentation that was successfully implanted, and a healthy baby was born.

References

1. Giorgetti C et al. Embryo score to predict implantation after *in-vitro* fertilization: based on 957 single embryo transfers. Hum Reprod. 1995;10(9):2427-31.
2. Fugimoto VY et al. Pathogenesis developmental consequences, and clinical correlations of human embryo fragmentations. Fertil Steril Rev. 2011;95(4):1197-204.
3. Yu C et al. A predictive model for high quality blastocyst based on blastomere numbers, fragmentation, and symmetry. J Assist Reprod Genet. 2018;35(5):809-16.

Efficacy of Endometrial Microbiome Metagenomic Analysis and Analysis of Infectious Chronic Endometritis in *In Vitro* Fertilisation Outcome in Women with Recurrent Implantation Failure

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Keywords: Analysis of infectious chronic endometritis (ALICE), chronic endometritis, endometrial microbiome metagenomic analysis (EMMA), *lactobacillus*, recurrent implantation failure (RIF).

Citation: EMJ Repro Health. 2020;6[1]:27-29. Abstract Review No: AR3.

BACKGROUND AND AIMS

Study Question

Following testing via endometrial microbiome metagenomic analysis (EMMA) and analysis of infectious chronic endometritis (ALICE) for patients with recurrent implantation failure (RIF), is there any positive impact identified after the suggested treatments?

Study Answer

The clinical pregnancy rate of the patients who underwent EMMA/ALICE testing was significantly higher than that of the patients who did not undergo testing.

What Is Already Known

Chronic endometritis is persistent endometrial inflammation, mainly caused by bacterial infections. Chronic endometritis is found in approximately 30% of women with infertility,[1,2] and 60% of patients with RIF;[3] pathogenic flora identification is the first step of treatment. Using next-generation sequencing technology, EMMA/ALICE testing can determine the composition of the endometrial microbiome by analysing bacterial *16S* ribosomal RNA with a focus on *Lactobacillus* population. *Lactobacillus*-dominated microbiota (LDM, defined as >90% *Lactobacillus* species) in the endometrium was reported to be associated with favourable reproductive outcome, while patients with non-LDM (<90% *Lactobacillus* species) endometrium were found to have decreased rates of implantation, clinical pregnancy, and ongoing pregnancy.[4]

Table 1: Patient characteristics and pregnancy outcomes.

	Patients with EMMA/ALICE (study group) (n=107)	Patients without EMMA/ ALICE (control group) (n=51)	p value
Age (years)	36.79±3.75	37.98±4.01	0.071
BMI (kg/m^2)	21.68±2.60	21.96±2.98	0.55
Duration of infertility (months)	49.36±32.81	40.25±32.67	0.104
History of delivery	24 (22.4%)	15 (29.4%)	0.341
History of miscarriage	51 (47.7%)	23 (45.1%)	0.091
Mean number of previous embryo transfer cycles	5.13±2.78	5.53±2.31	0.376
Transferred embryos (n)	1.39±0.49	1.41±0.57	0.827
Implantation rate (%)	77.6% (83/107)	47.0% (24/51)	<0.001
Clinical pregnancy rate (%)	64.5% (69/107)	33.3% (17/51)	<0.001
Ongoing pregnancy rate (%)	55.1% (59/107)	29.4% (15/51)	0.001
Multiple pregnancy rate (%)	11.9% (7/59)	6.7% (1/15)	0.686
Biochemical pregnancy rate (%)	13.1% (14/107)	13.7% (7/51)	0.141
Early miscarriage rate (%)	13.0% (9/69)	11.8% (2/17)	0.626

ALICE: Analysis of infectious chronic endometritis; EMMA: endometrial microbiome metagenomic analysis.

METHODS AND MATERIALS

Study Design, Size, and Duration

The prospective cohort study consisted of 158 females with RIF, defined as at least three previous failed *in vitro* fertilisation–embryo transfer (ET) attempts, from July 2018 to March 2020 at one infertility centre in Japan. EMMA/ALICE testing were suggested to all patients who had failed ET three or more times. The study group of 107 patients underwent EMMA/ALICE before additional transfer, while 51 patients with history of RIF continued with ET without these tests, to make up the control group.

Participants/Materials, Setting, and Methods

During the patients' luteal phase, endometrial biopsies were performed for EMMA/ALICE testing, and treatment was provided based on the results.

The primary outcome measure was the cumulative clinical pregnancy rate after two additional ET. Clinical pregnancy was defined by visualisation of a gestational sac. Statistical analysis was performed using unpaired t-test and chi-square contingency.

RESULTS

Patients' demographic data, such as age, BMI, duration of infertility, and anti-Müllerian hormone, were comparable between the groups. The mean numbers of implantation failure were also comparable between the groups (study group: 5.11±2.79 versus control group: 5.50±2.26). According to the results of EMMA, 52 patients (49%) with normal microbiota (i.e., LDM) did not receive probiotic treatment; the other 55 (51%) patients received probiotic therapies. In addition, among non-LDM patients, dysbiotic microbiota were detected in 23 patients (21%) by EMMA, and ALICE detected significant amounts of pathogenic

bacteria in 9 patients (corresponding to 39% of the patients with abnormal EMMA). Those 23 patients received antibiotic therapy according to the detected bacteria, followed by probiotic treatments. The cumulative clinical pregnancy rate in the study group was significantly higher than in the control group (study group: 64.5% versus control group: 33.3%). The difference in the implantation rate and the ongoing pregnancy rate were statistically higher in the study group than in the control group (Table 1).

CONCLUSION

Limitation

The main limitation of the study was the lack of randomisation, although it was prospective.

Wider Implication

Next-generation sequencing technology revealed that >50% of the patients studied had dysbiotic microbiota. Personalised treatment recommendations based on the EMMA/ALICE results can improve *in vitro* fertilisation outcome of patients who have experienced RIF and/or repeated pregnancy loss. Moreover, broad-spectrum antibiotic treatments can be avoided, reducing the physical and economic burdens on patients.

References

1. Johnston-MacAnanny EB et al. Chronic endometritis is a frequent finding in women with recurrent implantation failure after *in vitro* fertilization. Fertil Steril. 2010;93(2):437-41.
2. Kitaya K et al. Chronic endometritis: potential cause of infertility and obstetric and neonatal complications. Am J Reprod Immunol. 2016;75(1):13-22.
3. Cicinelli E et al. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. Reprod Sci. 2014;21(5):640-7.
4. Moreno I et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol. 2016;215(6):684-703.

Polycystic Ovary Syndrome as an Independent Risk Factor for Gestational Diabetes and Hypertensive Disorders of Pregnancy: A Population-Based Study of 9.1 Million Pregnancies

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Gestational diabetes (GDM), gestational hypertension (GHTN), polycystic ovary syndrome (PCOS), pre-eclampsia (PEC), pregnancy.

Citation: EMJ Repro Health. 2020;6[1]:29-32. Abstract Review No: AR4.

BACKGROUND AND AIMS

Currently, there is significant evidence of an increased prevalence of maternal pregnancy complications in women with polycystic ovary syndrome (PCOS).[1-4] However, there remain significant gaps in the understanding of how PCOS affects the development of gestational diabetes (GDM), gestational hypertension (GHTN), and pre-eclampsia (PEC).[5,6] This is most likely because of the complex, multifactorial aetiology of PCOS, its range of potential confounders for pregnancy complications, and the variable methodology of studies that have been conducted.

Table 1: Pregnancy outcomes of pregnant women with and without polycystic ovary syndrome, stratified by single and multiple gestations.

Pregnancy Complication	Total N=9,096,788 (%)					Singleton Pregnancies n=8,959,485 (%)					Multiple Pregnancies n=137,303 (%)				
	PCOS n=14,882	Non-PCOS n=9,081,906	Crude OR (95% CI)	Adjusted OR† (95% CI)	Adjusted p-value	PCOS n=14,002	Non-PCOS n=8,945,483	Crude OR (95% CI)	Adjusted OR† (95% CI)	Adjusted p-value	PCOS n=880	Non-PCOS n=136,423	Crude OR (95% CI)	Adjusted OR† (95% CI)	Adjusted p-value
Hypertensive disorders of pregnancy*	16.1	7.5	2.41 (2.32–2.54)	1.38 (1.27–1.50)	<0.001	15.6	7.2	2.37 (2.26–2.46)	1.41 (1.29–1.54)	<0.001	25.1	18	1.53 (1.31–1.78)	1.92 (0.99–1.42)	0.058
Gestational hypertension	6.6	3.3	2.07 (1.94–2.20)	1.47 (1.31–1.64)	<0.001	6.6	3.3	2.08 (1.94–2.22)	1.48 (1.31–1.66)	<0.001	6.9	5	1.43 (1.10–1.86)	1.22 (0.98–1.51)	0.21
Pre-eclampsia	7.1	3.6	2.04 (1.91–2.17)	1.29 (1.14–1.45)	<0.001	6.5	3.5	1.95 (1.83–2.09)	1.31 (1.15–1.50)	<0.001	15.1	12	1.31 (1.09–1.58)	1.22 (0.98–1.51)	0.75
Eclampsia	0.1	0.1	1.23 (0.73–2.08)	1.47 (0.55–3.95)	0.44	0.1	0.1	1.34 (0.79–2.53)	1.71 (0.64–4.60)	0.23	0.0	0.2	0.00	0.00	0.99
Superimposed pre-eclampsia/ eclampsia	2.7	0.5	5.31 (4.81–5.87)	1.29 (1.04–1.59)	0.02	2.6	0.5	5.26 (4.74–5.84)	1.34 (1.07–1.67)	0.01	4.0	1.2	3.36 (2.39–4.73)	1.28 (0.78–2.08)	0.33
Gestational diabetes	18.7	5.7	3.78 (3.63–3.94)	2.19 (2.02–2.37)	<0.001	18.4	5.7	3.73 (3.57–3.89)	2.17 (1.99–2.35)	<0.001	23.5	8.0	3.52 (3.02–4.12)	2.33 (1.92–2.83)	<0.001

*Previously referred to as pregnancy-associated hypertension.

†Adjusted for age, race, income quartile, insurance plan type, hospital type, obesity, smoking, use of *in vitro* fertilisation, previous caesarean section, chronic hypertension, pregestational diabetes, pre-existing thyroid disease, and maternal drug use.

CI: confidence interval; OR: odds ratio; PCOS: polycystic ovary syndrome.

To date, the largest meta-analysis on this subject includes 11,565 women with PCOS analysed for their risk of GDM and 5,896 patients analysed for their risk of PEC.[2] Unfortunately, the study was unable to isolate PCOS as an independent risk factor for PEC and other hypertensive disorders of pregnancy.

The objective of this study, therefore, was to determine if PCOS confers an independent risk for the development of GDM, GHTN, and PEC, utilising the Healthcare Cost and Utilization Project Nationwide Inpatient Sample (HCUP-NIS) database. The HCUP-NIS is the largest inpatient sample database in the USA and is comprised of hospital inpatient stays submitted by hospitals throughout the country. Each year, the database provides information relating to 47 million inpatient stays, including patient characteristics, diagnosis, and procedures. The data are representative of approximately 20% of admissions to USA hospitals and represents >96% of the American population.[7]

MATERIALS AND METHODS

This was a retrospective, population-based cohort study utilising data from the HCUP-NIS database over 11 years from 2004 to 2014. A cohort of all deliveries between 2004 and 2014, inclusive, was created. Within this group, all deliveries to women with PCOS were identified as part of the study group (n=14,882), and the remaining deliveries were categorised as non-PCOS births and comprised the reference group (n=9,081,906). The primary outcome of this study was the prevalence of GDM, GHTN, and PEC in pregnancies of women with PCOS compared to those without PCOS.

RESULTS

At baseline, more pregnant women with PCOS were obese (22.3% versus 3.5%; p<0.001), had chronic hypertension (8.4% versus 1.8%; p<0.001), had pregestational diabetes (4.1% versus 0.9%; p<0.001), and had treated thyroid disease (12.6% versus 2.4%; p<0.001). Women with PCOS were also more likely to have undergone *in vitro* fertilisation (IVF) treatment (2.4% versus 0.1%; p<0.001), have multigestation pregnancies (5.9% versus 1.5%; p<0.001), and more multiple gestations in the PCOS cohort were the result of IVF treatment than the non-PCOS cohort (12.3% versus 2.3%; p<0.001).

In all pregnancies, women with PCOS were more likely to develop GDM (adjusted odds ratio [aOR]: 2.19; 95% confidence interval [CI]: 2.02–2.37), pregnancy-associated hypertension (aOR: 1.38; 95% CI: 1.27–1.50; p<0.001), GHTN (aOR: 1.47; 95% CI: 1.31–1.64), PEC (aOR: 1.29; 95% CI: 1.14–1.45), and superimposed PEC (aOR: 1.29; 95% CI: 1.04–1.59) after controlling for confounding effects (age, race, income level, insurance type, obesity, IVF use, previous caesarean section, chronic hypertension, pregestational diabetes, thyroid disease, multiple gestation, smoking, and recreational drug use). Odds ratios were comparable between all pregnancies and singleton pregnancies only. In women pregnant with multiple fetuses, PCOS only conferred a statistically significant increased risk of developing GDM (aOR: 2.33; 95% CI: 1.92–2.83; p<0.001). However, there was a trend towards an increased risk for developing pregnancy-associated hypertension (aOR: 1.92; 95% CI: 0.99–1.42; p=0.058) (Table 1).

CONCLUSION

After controlling for all potential confounding effects, women with PCOS are at a two-fold higher risk of developing GDM, a 50% increased risk for the development of GHTN, and a 30% increased risk of developing PEC than women without PCOS. When caring for pregnant women with PCOS, it is important to also consider the risk of all other coexisting metabolic conditions frequently encountered in women with PCOS, as these risks are additive and place women with PCOS at significantly increased risk for adverse complications in pregnancy.

References:

1. Boomsma CM et al. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12(6):673-83.
2. Khomami MB et al. Increased maternal pregnancy complications in polycystic ovary syndrome appear to be independent of obesity-a systematic review, meta-analysis, and meta-regression. Obes Rev. 2019;20(5):659-74.
3. Qin JZ et al. Obstetric complications in women with polycystic ovary syndrome: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2013;11:56.
4. Kjerulff LE et al. Pregnancy outcomes in women with polycystic ovary syndrome: a metaanalysis. Am J Obstet Gynecol. 2011;204(6):558.
5. Khomami MB et al. Polycystic ovary syndrome and adverse pregnancy outcomes: current state of knowledge, challenges and potential implications for practice. Clin Endocrinol (Oxf). 2018;88(6):761-9.
6. Palomba S et al. Pregnancy complications in infertile patients with polycystic ovary syndrome: updated evidence. Minerva Ginecol. 2018;70(6):754-60.
7. Moore JE et al. Complicating conditions associated with childbirth, by delivery method and payer, 2011: Statistical Brief #173. Healthc Cost Util Proj Stat Briefs. 2006:1-19.

Pregnancy, Delivery, and Neonatal Outcomes Among Women with Congenital Adrenal Hyperplasia. A Population Study of Over 9 Million Patients

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Keywords: Chorioamnionitis, congenital adrenal hyperplasia (CAH) disease, congenital malformation, small gestational age.

Citation: EMJ Repro Health. 2020;6[1]:32-33. Abstract Review No: AR5.

BACKGROUND AND AIMS

Congenital adrenal hyperplasia (CAH) is a cluster of inherited enzymatic defects of adrenal steroid biosynthesis. Deficiencies of each enzyme required in the steroid biosynthesis pathway are well known, and these deficiencies are all inherited as autosomal recessive disorders. Women with CAH have decreased fertility because of oligo-ovulation. Conception requires a combination of proper therapeutic compliance, careful endocrine monitoring, and often ovulation induction.[1] There are significant gaps about pregnancy, delivery, and neonatal outcomes among CAH patients. The purpose of this study was to investigate these outcomes.

The aim of this study was to investigate the association between CAH and pregnancy, delivery, and neonatal outcomes, using a population database cohort.

MATERIALS AND METHODS

The authors conducted a retrospective study utilising the Health Care Cost and Utilization Project-Nationwide Inpatient Sample database from 2004–2014. ICD-9 code 255.[2] was used to extract the cases of CAH. Pregnancies complicated with CAH were compared to the other pregnancies. All confounding variables were adjusted for using multivariate logistic regression, based on any significant differences between the two groups.

RESULTS

9,094,499 deliveries occurred during the study period; 299 pregnant women had CAH. Chorioamnionitis was higher in patients with CAH compared to controls after controlling for risk factors (adjusted odds ratio [aOR]: 2.67, 95% [confidence interval] CI: 1.17–6.06). The rates of caesarean section and maternal infection were also higher in patients with CAH than controls (aOR: 2.10, 95% CI: 1.44–3.07, and aOR: 2.63, 95% CI: 1.22–5.63, respectively). Risk of gestational diabetes and pregnancy-induced hypertension rates were not increased in patients with CAH (aOR: 1.53, 95% CI: 0.91–2.58, and aOR: 0.87, 95% CI: 0.49–1.56, respectively).

At birth, 8% and 2.2% of the neonates were found to be small for their gestational age in the CAH and the control groups, respectively (aOR: 3.37, 95% CI: 1.86–6.11). Congenital anomalies were encountered in 2.7% and 0.4% in the CAH and control groups, respectively (aOR: 5.24, 95% CI: 2.31–11.90).

CONCLUSION

CAH patients were at risk of complications and fetal anomalies. Expected increases in rates of hypertension and gestational diabetes were not encountered. These patients will benefit from surveillance to decrease morbidity.

References:

1. Chatziaggelou A et al. Assisted reproduction in congenital adrenal hyperplasia. Front Endocrinol (Lausanne). 2019;10:723.

DNA Methylation Patterns Within Whole Blood of Adolescents Born from IVF Are Not Different from Those Adolescents Born from Natural Conception

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Keywords: DNA methylation, intracytoplasmic sperm injection (ICSI), *in vitro* fertilisation (IVF).

Citation: EMJ Repro Health. 2020;6[1]:33-35. Abstract Review No: AR6.

BACKGROUND AND AIMS

Epigenetics is defined as the study of mechanisms that control gene expression in a mitotically-heritable manner and are influenced by genetic, environmental, and developmental factors.[1] Growing evidence has suggested that the adverse health outcomes reported in IVF-born offspring might have underlying epigenetic mechanisms. Both the features of an infertile

couple, as well as the IVF procedure itself, have been shown to alter the epigenetic signature in the offspring and placental tissue.[2] As most studies have investigated, DNA methylation changes in cord blood/placental tissue[3-5] and a recent study reported that these changes are mitigated by adulthood.[6] It is essential to further investigate the potential effects of IVF on the DNA methylation profiles in adolescents using whole blood and to compare the findings to a large representative control group. In this study, the authors had a unique opportunity to investigate the differences in the epigenetic markers between the IVF-conceived adolescents from the Growing Up Healthy Study (GUHS) cohort and their naturally conceived, age-matched counterparts from the Raine study by comparing their DNA methylation signature (epigenomes) using epigenome-wide association studies (EWAS).

MATERIALS AND METHODS

Genomic DNA from whole blood was used to generate the epigenome profiles of the adolescents from the GUHS cohort using the Infinium Methylation Epic Bead Chip (Illumina Inc., San Diego, California, USA) which measures and quantifies approximately 850,000 DNA methylation probes. The epigenomes of the Raine cohort participants were generated from whole blood using the Illumina 450K array (Illumina Inc., San Diego, California, USA). The authors employed three analytical methods: EWAS, gene set enrichment analysis (GSEA), and four measures of epigenetic age. Differential DNA methylation differences between participants in the Raine and GUHS cohorts at age 17 were investigated followed by GSEA. Furthermore, differences in the methylation signatures between IVF and intracytoplasmic sperm injection (ICSI) offspring and frozen versus fresh embryo transfers within the GUHS cohort were investigated. GSEA and comparisons versus chronological age were also explored within the IVF cohort. The authors tested for an association between the cohorts applying Firth's bias reduced logistic regression against the outcome of IVF versus naturally conceived between the Raine study and GUHS. The effect of IVF on DNA methylation levels of 238 IVF-born adolescents, mean age 16.06±1.67 years (52.94% male), was compared to 1,188 naturally conceived, age-matched controls, mean age 17.25±0.58 years (50.93% male), from the Raine study. Results across all EWAS analyses were investigated to identify enriched biological pathways amongst the most significantly altered probes. Additionally, within the GUHS cohort, the authors investigated 792,104 DNA methylation probes for difference in methylation status applied GSEA to identify enriched pathways and compared four estimates (Horvath, Hannum, Levine, and Skin Horvath) of epigenetic age and their correlation with chronological age.

RESULTS

Between the two cohorts, a total of 401,022 DNA methylation probes overlapped. After adjustment for batch effects, DNA methylation probes as well as technical variation caused by different methylation platforms used between studies, none of the compared probes reached a Bonferroni correction of 1.24E-0.7 (0.05/402,022) required for statistical significance of a positive correlation. Of the analysed DNA methylation probes, 3,850 (0.96%), a small minority, showed nominal significance with a p value <0.05, most likely to be false positives after controlling for cross-study comparisons. Between the cohorts, 1,810 differentially methylated regions were identified; however, none reached statistical significance after correcting for multiple testing. In the comparison between Raine study and GUHS participants no significant enriched gene pathways were identified.

Within the GUHS cohort, when comparing the IVF versus ICSI offspring, and after adjusting for age, sex, maternal smoking, multiple births, batch effect, and cell type, the authors identified 5 CpG probes (*cg 15016734*; *cg 26744878*; *cg 0331628*; *cg 20235051*; *cg20233073*) that reached a Bonferroni correction of 6.31E-8. A further 20 probes were identified at a false discovery rate of 5%. Within the IVF cohort, the functional GSEA identified the neuroactive ligand–receptor interaction pathway, which remained significant (p=0.00048) after adjusting for age and sex. In the analysis of epigenetic ageing, the authors found that all four measures were highly correlated with chronological age and did not demonstrate evidence of significant

accelerated ageing within the GUHS cohort. The Levine method provided the weakest correlation for accelerated ageing (r^2=0.23) and Skin Horvath had the best fit (r^2=0.61), followed by Horvath (r^2=0.35) and Hannum (r^2=0.28).

CONCLUSION

The authors observed no significant differences in the DNA methylation profiles of adolescents born from IVF when compared to their naturally conceived, age-matched counterparts. A statistically significant difference in the methylation profiles was identified within the IVF cohort when comparing the IVF and ICSI-conceived offspring. Furthermore, an enriched gene pathway among the altered methylation profiles was identified. The IVF cohort showed no evidence of accelerated epigenetic ageing within their whole blood.

References

1. Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. Nature. 2019;571(7766):489-9.
2. Jiang Z et al. Genetic and epigenetic risks of assisted reproduction. Best Pract Res Clin Obstet Gynaecol. 2017;44:90-104.
3. El Hajj N et al. DNA methylation signatures in cord blood of ICSI children. Hum Reprod. 2017;32(8):1761-9.
4. Estill M et al. Assisted reproductive technology alters deoxyribonucleic acid methylation profiles in bloodspots of newborn infants. Fertil Steril. 2016;106(3):629-39.
5. Ghosh J et al. Global DNA methylation levels are altered by modifiable clinical manipulations in assisted reproductive technologies. Clin Epigenetics. 2017;9:14.
6. Novakovic B et al. Assisted reproductive technologies are associated with limited epigenetic variation at birth that largely resolves by adulthood. Nat Commun. 2019;10(1):3922.

Local Steroid Metabolism in Eutopic Endometrium and Corresponding Endometriotic Lesions: Intra-Patient Variability

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Keywords: Androgens, corticosteroids, endometriosis, intracrinology, local steroid metabolism, oestrogens, progestogens.

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BACKGROUND AND AIMS

Endometrial tissue produces steroids locally that can be relevant in endometriosis;[1] oestrogens control lesion establishment, progestogens oppose oestrogen action, androgens are oestrogen precursors, and corticosteroids suppress inflammation. To elucidate to what extent steroid metabolism is implicated in endometriosis, and what inter- and intra-patient

variability exists, the authors profiled major steroids in blood and tissue (normal endometrium and endometriosis) of patients, and also determined the expression levels of major enzymes involved in local steroid metabolism.

MATERIALS AND METHODS

This was a retrospective study using biobanked frozen patient material. Eutopic endometrium, multiple endometriotic lesions from each patient, and peripheral blood of 14 women (seven in the luteal and seven in the follicular phase) with histologically confirmed endometriosis were analysed. Endometriotic lesions originated from the uterosacral ligament/Pouch of Douglas, bladder, ovarian fossa, and rectum/rectosigmoid. Patients had Stage I (n=1), II (n=9), III (n=3), or IV (n=1) endometriosis (American Society for Reproductive Medicine [ASRM] classification). Patients with endometriosis were not under hormonal medication for six months prior to the biopsy. Plasma, eutopic endometrium samples (n=14), and endometriotic lesions (n=39) were obtained and stored following the Endometriosis Phenome and Biobanking Harmonisation Project (EPHect)/World Endometriosis Research Foundation (WERF) guidelines.[2] RNA expression was determined by whole RNA sequencing. Levels of major steroids were measured by liquid chromatography-mass spectrometry (LC-MS).[3] HSD17B1 activity was measured in cell-free extracts by high-performance liquid chromatography (HPLC).[4,5]

RESULTS

Oestrogens (oestrone, oestradiol) were non-statistically significantly higher in eutopic and endometriotic tissues compared with blood (oestradiol: 1.0 pmol/g eutopic; 3.2 pmol/g endometriotic; 0.4 pmol/mL blood; and oestrone: 0.3 pmol/g eutopic; 1.1 pmol/g endometriotic; 0.3 pmol/mL blood). Of note, oestradiol:oestrone ratios, approximately 1 in blood, are approximately 3 in tissue, indicating active local synthesis. 17-hydroxy-progestogens and androstenedione were over four-fold higher in endometriotic lesions than eutopic tissue ($p<0.05$). The activity of HSD17B1 was comparable between eutopic and endometriotic tissues.

Regarding corticosteroids, active cortisol was four-fold higher in endometriosis than in the eutopic tissue ($p<0.001$), whereas inactive cortisone was 2.5-fold lower in endometriosis ($p<0.001$). HSD11B1 (activation to cortisol) and HSD11B2 (deactivation to cortisone) mRNA levels were in line with the corticosteroid levels; HSD11B1 mRNA was higher in endometriosis, and the opposite was observed for HSD11B2 compared with the eutopic endometrium ($p<0.001$ for both enzymes). The levels of compounds acting as precursors for corticosteroid synthesis (i.e., 11-deoxycortisol, 11-deoxycorticosterone) were higher in endometriosis compared with the eutopic tissue ($p<0.05$), and a number of enzymes involved in the generation of active compounds from these precursors were expressed in both eutopic endometrium and endometriotic tissue.

CONCLUSION

Although this was a retrospective study and included patients with all stages of disease and with manifestation of different symptoms in a pooled analysis, these data show that steroid levels differ between normal and endometriotic tissue. Irrespective of the location, endometriosis shows active synthesis of oestrogens and sustained corticosteroid levels.

References

1. Konings G et al. Intracrine regulation of estrogen and other sex steroid levels in endometrium and non-gynecological tissues; pathology, physiology, and drug discovery. Front Pharmacol. 2018;9:940.
2. World Endometriosis Research Foundation. WERF Endometriosis Phenome and Biobanking Harmonisation Project (EPHect). 2019. Available at: https://endometriosisfoundation.org/ephect/. Last accessed: June 2020.
3. Tangen I et al; ENITEC. Blood steroids are associated with prognosis and fat distribution in endometrial cancer. Gynecol Oncol. 2019;152(1):46-52
4. Delvoux B et al. Inhibition of type 1 17beta-hydroxysteroid dehydrogenase impairs the synthesis of 17beta-estradiol in endometriosis lesions. J Clin Endocrinol Metab. 2014;99(1):276-84
5. Delvoux B et al. Increased production of 17beta-estradiol in endometriosis lesions is the result of impaired metabolism. J Clin Endocrinol Metab. 2009;94(3):876-83.

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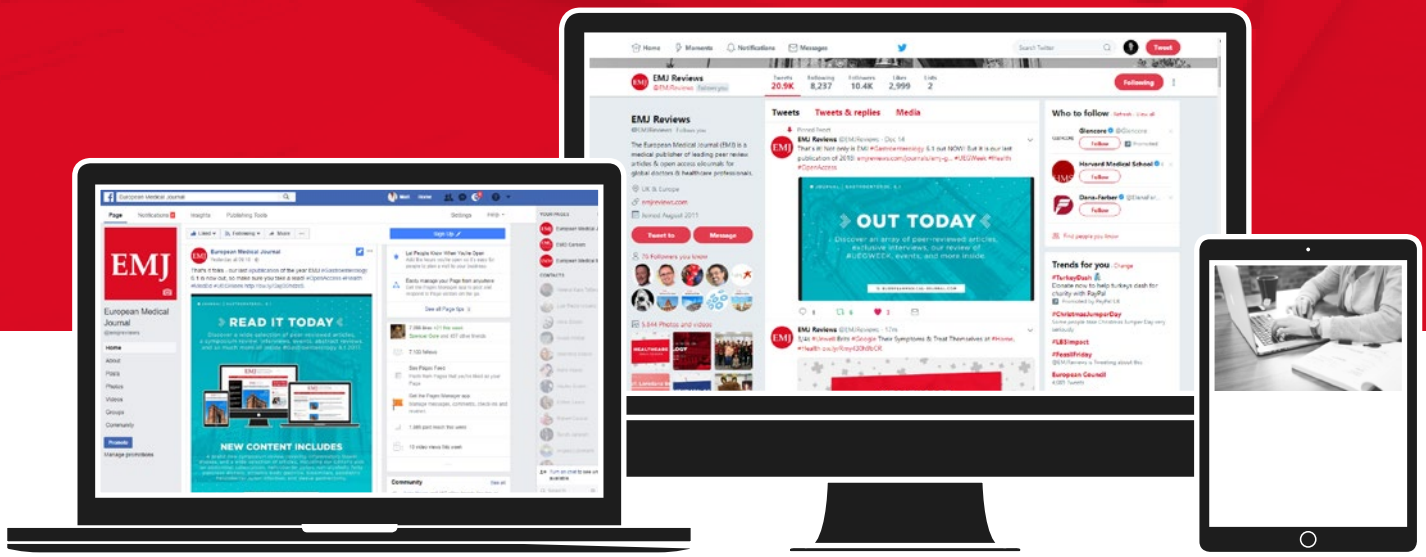

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Interview

Prof Andrew Shennan

Professor of Obstetrics, King's College London, St Thomas' Hospital, London, UK

Q1

Was there a particular event or person that inspired you to pursue a career in obstetrics?

I think it was a slow but very focussed realisation for me that this specialty would be my choice. Interested, passionate teachers implanted the seed. Then individuals with enthusiasm showed me how rewarding and worthwhile it could be. I have tried to emulate them. A medical career can be hard, and you hear a lot of negativity, particularly in a high-risk, 24/7 craft specialty with medicolegal pressures. The emotions in the specialty can be extreme but I have had no regrets about my choices.

Q2

Could you enlighten us on the overall mission of the National Institute for Health Research (NIHR)? You have been Reproductive Health and Childbirth Speciality Group National Chair, and are now the Director for the South London Clinical Research Network (CRN) and a national spokesperson.

Simply, the NIHR aims to improve the health and wealth of our nation. We have focussed on embedding research throughout the National Health Service (NHS) and social care. This unique research system in the UK has been very successful. The reproductive health specialty now recruits more participants to trials than any other specialty, in spite of women's health previously having been a relatively poor relation in research. We now try to mimic this success across all specialties and, although South London is small, I'm proud to say that in 2020 we are the epicentre of research activity in the UK, thanks to all the CRN staff and leading on delivering the COVID-19 studies.

Q3

You are involved with various charities, such as Action on Pre-eclampsia (APEC); Tommy's, the Baby Charity; and Action Medical Research. Has advocacy in patient care always been a passion of yours?

Academic clinicians have a very privileged position in having time to evaluate and disseminate knowledge. However, uptake and implementation of research findings into clinical practice are equally important but not usually our remit. Delays in introducing innovation are all too common. The charity sector has an important role to play in bridging these gaps, understanding the user perspective, informing the research questions and delivery, and then presenting the findings to those who matter. I think it is an essential component of my job and allows me to engage with people from a more diverse background and

understand the wider world. In short, charitable work aids in helping others; that's why we are in this business!

Q4

Your current research interests include pre-eclampsia and interventions to predict and prevent preterm birth. What other areas of obstetrics do you believe merit wider attention?

As I get older and feel time is running out, you focus on the big questions. Global maternal mortality is probably the biggest, easily reversed injustice I can think of. In many countries it is hundreds of times greater than it could be, a reduction that could be achieved with relatively simple action. Bleeding, usually after delivery, is perhaps the biggest cause. My pre-eclampsia research has now branched out into detecting and managing haemorrhage in low-income settings. Simple things can make a big difference.

Q5

The most effective treatment for pre-eclampsia is the delivery of the baby; however, what treatment options are available for postpartum pre-eclampsia?

The paradox here is that timely delivery is also key to preventing serious complications with postpartum pre-eclampsia. The disease will go away with delivery, just not immediately. Our challenge is finding markers that allow us to justify earlier delivery, but not to do an unnecessary early delivery. We have also shown that routine delivery from 34 weeks in women who have pre-eclampsia in the UK may be justified (the PHOENIX trial), and our current work is evaluating this in low-income settings where the advantage of this could be much greater (CRADLE-4 trial).

Q6

In your opinion, what are the most significant breakthroughs and challenges you have seen in obstetrics during your time working in the field?

Remarkable developments have been seen in prenatal diagnosis in recent decades. Technology now allows us to rapidly detect abnormal babies, even through maternal blood samples. Options remain crude though, i.e., usually to end the pregnancy.

Perhaps the most significant challenge has been our ability to detect fetal distress during labour. The poor monitoring, coupled with our increasingly medicolegal, risk-averse culture, means that the caesarean section rate has tripled in my working lifetime, with only marginal benefit, if any, to mother and baby. Indeed, emergency caesareans are now known to cause preterm labour in future pregnancies, for example. This is a challenge for the next generation.

Q7

What are the key take-home messages from your recently published paper 'Rule-in thresholds for DELFIA Xpress PlGF 1-2-3 test for suspected pre-eclampsia'?

This is one of the new blood tests that can accurately detect risk in women with suspected pre-eclampsia. We are fortunate that the NHS has decided to roll these out nationally. Many women will present with mild symptoms and signs, and it is impossible to know if they will be seriously ill or not require delivery for some months. This simple blood test will allow this triage. In the post-COVID-19 world, this is even more important where we don't want unnecessary patient-facing episodes, but don't want to miss women at risk.

Q8

We see that you often give advice to international organisations such as the World Health Organization (WHO) and The International Federation of Gynaecology and Obstetrics (FIGO). What do you hope to achieve within these roles?

My interest in patient care is worldwide. The vast majority of birth complications (>90%) do not occur in the USA or Europe, yet this is where most guidelines are from. These organisations (WHO/FIGO) tend to tailor their advice to the circumstances and resources in the real world where most women give birth. This is where we can make the biggest impact. I like to bring our sophisticated knowledge to those who really need it; the challenge is to learn from the rest of the world and build capacity and partnerships to

"The charity sector has an important role to play in bridging these gaps, understanding the user perspective, informing the research questions and delivery, and then presenting the findings to those who matter."

achieve this. My international research has helped this enormously and I have a wonderful network of friends and colleagues around the world now.

Q9 Congratulations on being appointed an Officer of the Order for the British Empire (OBE) for your services to maternity care in 2018! Could you tell us how your work in this area has progressed since then?

This was a delightful surprise, and a genuine honour for me. The work has continued by rolling out national guidelines in preterm birth management, supported by NHS England. Our trials continue to fine-tune new and promising interventions to predict and prevent preterm birth. We have launched an app for prediction of preterm birth, and have shown some procedures (e.g., abdominal cerclage) are a life saver. We are now finding ways to disseminate this more quickly, via video and teaching others.

Q10 In 2017 you were awarded the prestigious Newton Prize, a fund for excellence in research and innovation in support of academic development and social welfare. Please tell us more about your work in low- and middle-income countries?

You probably have gathered my real passion is global health. Many individuals contributed to the success of this prize. More than 35,000 of our CRADLE Vital Signs Alert (VSA) device (to detect shock and blood pressure) have been disseminated in over 40 countries, and we are developing its use outside pregnancy (e.g., to detect malaria in refugee camps in Uganda, and anaemia in India). 3,000 devices in Sierra Leone have been redeployed to help with the COVID-19 crisis.

"My interest in patient care is worldwide. The vast majority of birth complications (>90%) do not occur in the USA or Europe, yet this is where most guidelines are from."

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When Regenerative Medicine Faces the Challenges of Reproductive Medicine: A Review Study on Recent Advances in the Strategies for Derivation of Gametes from Stem Cells

EDITOR'S PICK

This comprehensive review by Gil Juliá and Medrano on recent advances on *in vitro* gametogenesis provides clues for what is still missing and what are the future steps to take to produce male and female gametes *in vitro* from stem cells. Clearly, the way ahead is still long and difficult, but the recent successes in cryopreservation of mature oocytes and ovarian tissue, and the birth of the first macaque conceived with spermatozoa obtained after transplantation of prepubertal testicular tissue, give hope for fertility preservation of prepubertal cancer patients.

Prof Elisabetta Baldi

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Abstract

The murine model has allowed for the replication of all developmental stages of the mammalian germline *in vitro*, from embryonic stem cells to epiblast cells, primordial germ cells, and finally into functional haploid gametes. However, because of interspecies differences between mice and humans, these results are yet to be replicated in our species. Reports on the use of stem cells as a source of gametes, retrieved from public scientific databases, were analysed and classified according to the animal model used, the stem cell source and type, the differentiation strategy, and its potential application. This review offers a comprehensive compilation of recent publications of key events in the derivation of germ cells and gametogenesis *in vitro*, in both mice and human models. Additionally, studies intending to replicate the different stages in human cells *in vitro*, in order to obtain cells with a phenotype akin to functional human gametes, are also depicted. The authors present options for deriving gametes from stem cells *in vitro* and different reproductive options for specific

groups of patients. Lastly, the potential applications of *in vitro* human gametogenesis are evaluated as well as the main limitations of the techniques employed. Even though it appears that we are far from being able to obtain gametes from pluripotent stem cells *in vitro* as a viable reproductive option, its current academic and clinical implications are extremely promising.

INTRODUCTION

Gametes are highly specialised cells that allow the birth of new individuals and, with that, the continuation of a species. Gametogenesis is a carefully orchestrated process in which primordial germ cells (PGC), the precursors of gametes, undergo specification, epigenetic reprogramming, and differentiation into functional adult gametes: spermatozoa and oocytes.[1,2] In this context, the use of stem cells to derive germ cells *in vitro* during the last decade has allowed us for the first time to study the development of germ cells. This has paved the way to understanding key events that may help develop new strategies to produce functional gametes for infertile patients, unable to produce them, and allow their genetic parenthood in the future.

The specification of the germline starts in the embryo with the derivation of PGC from epiblast cells and because this occurs during the implantation of the embryo, it is an inaccessible process for research in humans.[3] Therefore, the mechanisms involved in this process have been studied in different animal models, primarily mice.[4] Even though the common elements within the array of transcription factors that regulate the specification of human and mouse PGC have been described, significant differences call for the design of models for the development of the germline and *in vitro* gametogenesis from human cells (Figure 1).[5,6] The acquisition of the so-called 'germline fate' is the result of events such as the activation of the *BLIMP1/PRDM1* and *PRDM14* network by bone morphogenetic protein 4 (BMP4) produced by extraembryonic tissues,[7] the repression of the mesodermal fate, and re-establishment of pluripotency. This is pivotal to achieve epigenetic reprogramming,[1,6] as well as the expression of germline-specific factors PRDM1/BLIMP1, TFAP2C or DAZL, and pluripotency factors OCT4 and NANOG. However, key differences between the murine model and the human reside in how these factors interact and how they execute their function.[5] After their specification in the posterior region of the epiblast in response to the extraembryonic signals described above, PGC multiply and migrate towards the genital ridges as they reprogramme their epigenome, erasing almost all epigenetic marks in their DNA with the exception of some families of transposable elements to avoid the transmission of epigenetic mutations to the offspring and reset imprinting marks according to their sex. Following this, in a gonadal sex-dependent phase, germ cells undergo changes in their morphology and epigenome as they enter cell cycle arrest in meiosis prophase I (in the case of human female germ cells or oogonia) or in a quiescent premeiotic state (in the case of human male germ cells or gonocytes). It is their interaction with the gonadal niche that determines the continuation of meiosis and the remethylation pattern that will be followed after birth in oogonia or immediately after sex determination in gonocytes.[1-3]

Although our understanding of the molecular mechanisms involved in the production of human gametes is increasing, this information is still limited. It is mandatory to understand the specific events responsible for the derivation of human PGCS (hPGC) *in vivo* to be able to obtain hPGC-like cells (hPGCLC) from pluripotent stem cells in vitro[6] and exploit their clinical applications consciously and safely. This raises the need to develop *in vitro* gametogenesis models, not only to obtain immediate treatments for specific groups of patients, but also to perfect the newest protocols that are currently arising for their differentiation in the laboratory while ensuring that the end product is safe and guarantees success. The aim of this comprehensive review is to compile recent research publications on key events in the derivation of germ cells and the replication of gametogenesis *in vitro* in both mice and human models, assessing the limitations of these findings, and discussing their potential applications in reproductive medicine.

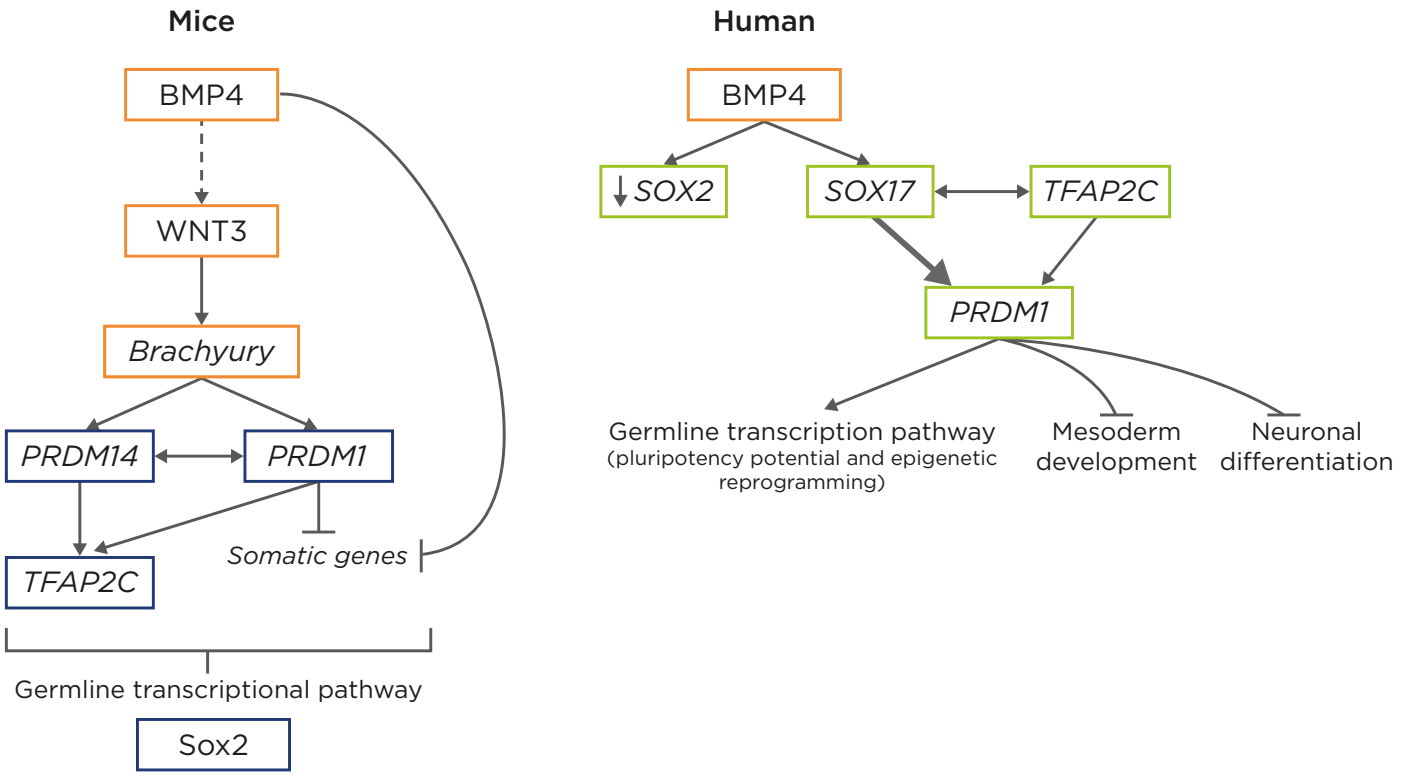

Figure 1: Differences in molecular mechanisms involved in the specification of mice primordial germ cells versus human primordial germ cells in early post-implantation embryos.[5,6]

In mice, extra-embryonic ectoderm primordial germ cells (PGC)-competent cells secrete bone morphogenetic protein 4, which directly or indirectly activates the Wnt3 signalling pathway in the epiblastic cells, from which the activation of brachyury results, driving the first steps of differentiation during gastrulation. Brachyury activates both PRDM14 and PRDM1 inside PGC, driving the activation of TFAP2C and the inactivation of somatic genes, which trigger the expression of germline-specific transcription factors. Thus, the upregulation of PRDM1, PRDM14, and TFAP2C would suffice to induce a PGC-like state from mice epiblastic cells. In mice, SOX2 is a crucial pluripotency factor, which will recover its importance during mouse PGC specification later on. In humans, PGC-competent cells in the posterior epiblast have an increased expression of secrete bone morphogenetic protein 4, which will activate SOX17 inside the subset of cells connected to the mesoderm developing into human PGC. The upregulation of SOX17 is accompanied by the downregulation of the pluripotency marker SOX2, since their expression is mutually exclusive in humans, representing one of the main differences between mice and human early germline development. Also, brachyury, which was a main driver of mouse PGC, is not essential as an activator in humans. The network formed by SOX17, PRDM1, and TFAP2C is the main initiator of human PGC fate by activating the germline-specific transcriptomic pathway and inhibiting both the mesodermic and neuronal differentiation programmes.

Adapted from Kobayashi et al.[5] and Saitou et al.[6]

RECONSTRUCTION OF THE PRIMORDIAL GERM CELLS SPECIFICATION *IN VITRO*

During the first decade of the 2000s, several reports focussed on the obtention of gametes *in vitro* from organotypic culture, either by maturation of fetal mice secondary ovarian follicles in culture from PGC to mature metaphase II oocytes,[8] or by retrieval of spermatogonial stem cells (SSC) from testicular biopsies.[9] Despite the improvements in organ cell culture becoming a valuable tool for fertility treatments, this review focusses on the replication of the process of germline development from embryonic stem cells to the obtention of gametes.

To develop a model for the derivation of the germline *in vitro*, it is key to accurately replicate the transition between the two phases of pluripotency, naïve and primed, providing the starting pluripotent cells with competence to be specified into PGC first, and then completing

their differentiation into PGC-like cells (PGCLC). Following this logic, Hayashi[10] et al. introduced a method to obtain mouse PGCLC (mPGCLC) from mice embryonic stem cells (mESC) in two steps. First they re-established the naïve pluripotent state of mESC using two kinase inhibitor cocktails and leukaemia inhibitor factor to start from a homogeneous pluripotent cell cohort[1] that would mimic the properties of cells found in the internal mass of blastocysts,[11] and pushed them to differentiate into epiblast-like cells (EpiLC) with competence to produce PGC. After that, using BMP4 plus a combination of other cytokines, the group achieved the differentiation of EpiLC into mPGCLC which were then transplanted into the mice testes to investigate if the cells were able to colonise the seminiferous tubules, complete meiosis, and mature. Once sperm were retrieved, they were used to fertilise oocytes by intracytoplasmic sperm injection (ICSI) and produced healthy offspring. This was the first evidence of the use of mice gametes obtained from pluripotent cells *in vitro* in assisted reproduction.[1,10] One year later, the group used the same technique, this time replicating oogenesis and obtaining functional oocytes from aggregates of mPGCLC with somatic cells from mice embryonic ovaries. These aggregates were transplanted into the ovarian bursa, generating structures similar to follicles and oocytes within them. After ICSI using these retrieved oocytes, healthy offspring were obtained. Unfortunately, the method was proven to have limited efficiency, since a large subset of oocytes derived from mPGCLC were unable to extrude the second polar body after fertilisation, approximately 53% of the resulting zygotes were tripronuclear and, of those with two pronuclei, 35% were dysgenic diploid zygotes, with two maternal pronuclei. Despite the aptitude for fertilisation of the resulting cells needing further investigation, these strategies set the foundation from which to attempt the reconstruction of the early stages of germline development in mammals.[12]

In humans, the two-step differentiation strategy has also been performed to obtain hPGC *in vitro* from human ESC (hESC) or human induced pluripotent stem cells (hiPSC). In 2015, Irie et al. cultured hESCwith a combination of 4 kinase inhibitors (4i) for the restoration of naïve pluripotency. EpiLC were also preinduced in response to BMP4 and derived into PGCLC, employing the same cytokine cocktail used by Hayashi et al. However, the authors noted that hPGCLC were able to derive directly from pluripotent cells in the 4i condition bypassing the preinduction state to EpiLC. Based on this, they concluded that the 4i state may be closer to the epiblast state than to a naïve pluripotent state, which explains why no preinduction was required.[13] In the same year, Sasaki et al.[14] achieved similar results when using hiPSC from a non-naïve prepared pluripotent state, obtaining incipient mesodermal-like cells, which were differentiated following the beforementioned protocol into hPGCLC.[14]

From the differentiation strategies described, countless advances in the characterisation of the molecular mechanisms involved in the first step of the germline generation (until the obtention of PGCLC) have been made. The crucial characterisation of the network formed by factors PRDM1/BLIMP1, PRDM14 and TFAP2C in germinal fate specification in mice,[15] the synergic action of SOX17 and PRDM1/BLIMP1, the progressive upregulation of NANOG and TFAP2C and downregulation of SOX2 in the human *in vitro* model,[16] the role of TFAP2C in maintaining an open chromatin site in the naïve pluripotency enhancer *OCT4* in hPGCs,[4] the importance of the Wnt signalling pathway stimulation,[11] and the dual behaviour of NANOG, PAX5, OCT4, and pRDM1 having more affinity to germline-specific genes in hPGCLC or pluripotency genes in hESC are a few examples of mechanisms involved in the early stages of gamete derivation (Figure 2).[4,13,16-18]

GAMETOGENESIS MODELS FOR THE *IN VITRO* OBTENTION OF GAMETES FROM PLURIPOTENT CELLS

As a result of the differentiation protocols described above, other research groups have studied the mechanisms of the specification of PGC and their derivation into SSC), or oogonial stem cells to a deeper level.

Spermatogenesis

In 2016, Zhou et al.[19] applied the aforementioned Hayashi's two-step differentiation protocol to mESC and mice IPSC and obtained mPGCLC that were coaggregated with somatic testicular cells

retrieved from mice carrying a mutation for *c-kit*, which produces a lack of endogenous germ cells. After the differentiation of the coaggregates in culture with follicle stimulating hormone, testosterone, bovine pituitary extract, BMP2 and BMP4, mPGCLC were able to progress through meiosis. Moreover, the haploid spermatid-like cells obtained presented the same imprint pattern as spermatids *in vivo*. Finally, spermatids were microinjected into oocytes, producing a healthy offspring.[19]

That same year, Ishikura et al.[20] used Irie's two-step differentiation protocol, but in this case, mPGCLC were further isolated and cultured in suspension in coaggregation with somatic cells retrieved from fetal mice testis (Table 1).[14,20-23] The majority of reconstituted testicular aggregates formed structures similar to seminiferous tubules with mPGCLC located within them. mPGCLC were subsequently differentiated into mouse germline stem cell-like cells (mGSCLC), which expressed pluripotency (tyrosine-protein kinase KIT), germinal differentiation (*DDX4*, *DAZL*, *SSEA1*), and spermatogonial (PLZF) markers, resembling the phenotype of mouse germline stem cells (mGSC) *in vivo*. mGSCLC were then transplanted into germ cell-depleted adult mice testes.

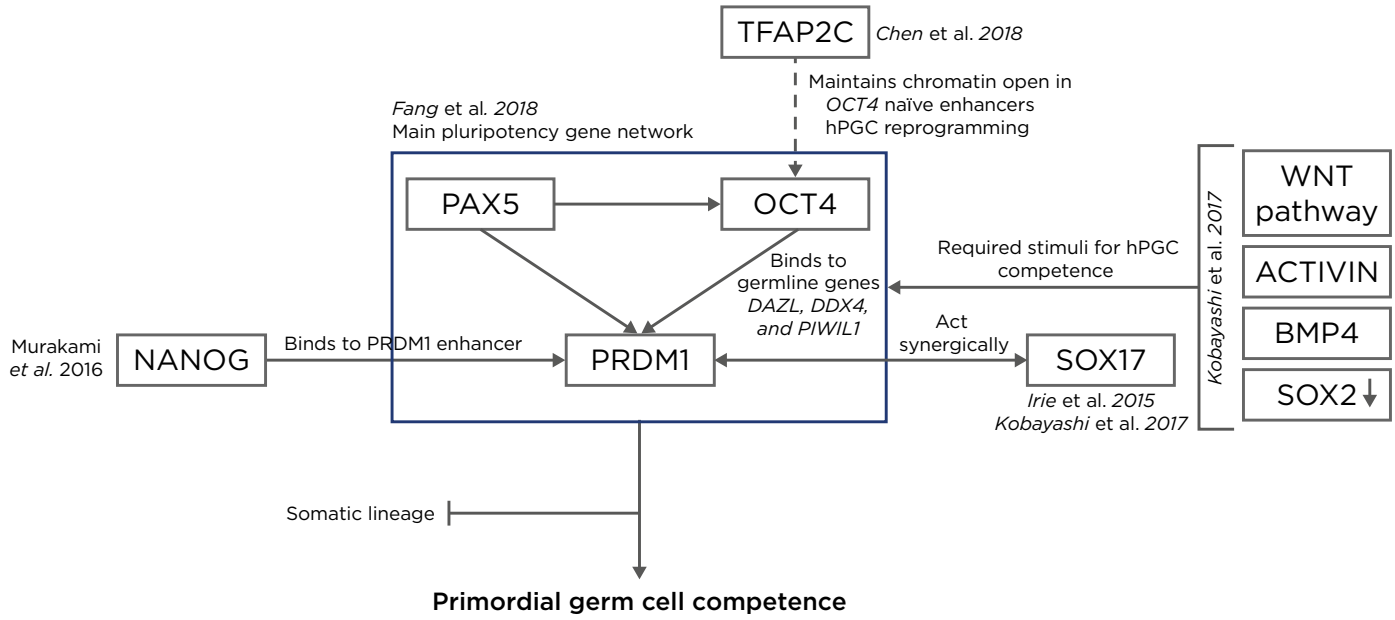

Figure 2: Proposal for the characterisation of the molecular mechanisms of the early differentiation of human embryonic stem cells (hESC) into human primordial germ cells (hPGC).[4,13,16-18]

SOX17 is key in the derivation of the human germline and the formation of the endoderm and it acts synergic with PRDM1 to confer pluripotent cells with primordial germ cell (PGC) competence,[13,16] together with stimuli from the Wnt signalling pathway, activin, bone morphogenic protein 4, and the downregulation of SOX2.[16] Murakami et al.[17] confirmed that NANOG was a key contributor to the germline fate of hESC while introducing that the role of that transcription could depend on the epigenetic context and stage. In mouse ESC, the role of NANOG is the maintenance of pluripotency, whereas in mouse PGC it binds to the enhancer region in PRDM1 to confer PGC competence.[17] However, this study uses the mouse model and the suggested mechanism must be validated in human cells to be definitively included in the differentiation network from hESC to human PGC. Fang et al.[18] studied the main pluripotency gene network in both hESC and hPGC. They suggested that PAX5 regulates the expression of OCT4 while they both act jointly over PRDM1 during PGC specification. The increase in expression of PRDM1 together with the binding affinity of OCT4 switching from pluripotent genes to germline-specific genes *DAZL, DDX4* and *PIWIL1*, were characteristic of hPGC.[18] In 2018, Chen et al.[4] noted that one of the roles of TFAP2C Is the opening of chromatin in naïve enhancers, one of them located in the OCT4 locus. The result is the induction of a brief second naïve pluripotency during which hPGC undergo epigenetic reprogramming.[4] The result of all these interactions is the suppression of those genes that would deem the cells to the somatic lineage and, instead, the conferral of the PGC competence in order to develop the germline.

Table 1: The most recent studies in the obtention of gametes or their precursor cells *in vitro*.[14,20-23] Studies from 2016 until February 2020 have been considered 'recent'. These publications were classified according to the process that they aimed to reproduce *in vitro* (spermatogenesis or oogenesis), the study model, the starting and resulting cells, a brief description of the methods used, the key markers that were assessed, and the main findings.

Process	Model	Starting cell	Study cell	Description	Main markers	Main conclusions	Reference
Spermatogenesis	Mice	mouse ESC	GSCLC with SSC ability SSC	Induction of mouse ESC into epiblast-like cells, then to PGLC Obtention of PGCLC reconstituted testicle (mouse PGCLC plus fetal testicle somatic cells), differentiation *in vitro* into GSCLC	DDX4, DAZL, KIT, PLZF, ID4, CD9, SSEA1, integrins β1 and α6	After GSCLC transplant into adult mice testicle, approximately 40% complete spermatogenesis. After ICSI/ROSI, healthy and fertile offspring is obtained. The majority of those that do not complete it and show demethylation and methylation errors in regulatory elements such as KIT.	Ishikura et al.,[20] 2016
	Rhesus macaque xenotransplant to mice; homologous transplant rhesus macaque	riPSC	PGCLC	Sasaki et al.,[14] 2015 2-step differentiation protocol	VASA, MAGEA4, 5mC, 5hmC, OCT4, TFAP2C, ENO2	Both in xenotransplant and homologous transplant, PGCLC start epigenetic reprogramming and differentiation to spermatogonia (VASA and MAGEA4 positive). However, they are not able to complete spermatogenesis (ENO2 negative).	Sosa et al.,[21] 2018
Oogenesis	Human cell line	human ESC	Oocytes	· Differentiation into PGC over feeder enriched with BMP4 and BMP8a · Induction of meiosis via overexpression of DAZL and BOULE · Induction of folliculogenesis using human recombinant GFP9 and BMP15	VASA, SYCP3, PRDM9, H2AX, ZP2, NOBOX	First study that showed the formation of structures similar to primary follicles from human ESC, which contain a structure similar to an oocyte with granulosa cells. No supporting gonadal tissue needed.	Jung et al.,[22] 2017
	Human cell line	hiPSC	Oogonia and gonocytes	·Induction of male and female hiPSC into iMeLC and human PGCLC. · Generation of reconstituted xenogenic cells by coaggregation of Day 6.0 human PGCLC with Day 12.5 mice embryo ovarian somatic cells	NANOG, PRDM1, TFCP2L1, TFAP2C, DAZL, DDX4, SYCP3, STRA8, 5mC	Proof that oogonia derived from hPGCLC cultured in a reconstituted ovary with mice ovarian cells have the ability to express germline genes, start epigenetic reprogramming, and the erasing of imprint. However, there are deficiencies in the reactivation of the inactive X chromosome.	Yamashiro et al.,[23] 2018

ESC: embryonic stem cell; GSCLC: germline stem cell-like cells; hiPSC: human induced pluripotent stem cells; iMeLC: incipient mesodermal-like cells PGCLC: primordial germ cell-like cell; riPSC: rat-induced pluripotent stem cell; SSC: spermatogonial stem cells.

However, only around 40% of them were able to complete spermatogenesis and produce healthy offspring after microinjection into oocytes and because most mGSCLC stopped at meiosis prophase I, the methylation patterns in distinct stages of the differentiation were studied, unveiling errors in DNA methylation during mPGCLC differentiation. Therefore, even though the ability of mGSCLC to complete spermatogenesis was limited compared to that of mGSC, this study developed a protocol by which mESC are induced into stable *in vitro* cell cultures with the mouse SSC ability to forerun spermatozoa and spermatids in adult mice testicles.

To date, most studies that focussed on targeted differentiation to generate male PGCLC *in vitro* have failed to showcase the expression of mature germ cell markers, which translates into PGCS in a very primary state. In 2018, Sosa et al.[21] and colleagues derived PGCLC *in vitro* using Sasaki's two-step differentiation protocol that expressed pluripotency markers SOX17, TFAP2C, PRDM1, and OCT4 (Table 1).[14,20-23] Once they were transplanted into previously sterilised mice of rhesus macaques testes, cells differentiated into a prespermatogonial phenotype, expressing VASA and MAGEA4. Furthermore, the expression of 5-hydroxymethylcytosine proved that the cells had commenced their epigenetic reprogramming. This study showed that physical contact between *in vitro*-induced PGCLC and the adult gonadal ridge is not required for them to differentiate. Nevertheless, both models of PGCLC transplantation halted the cell cycle and prevented their transition into spermatogonia.[21]

Oogenesis

Similar to the co-aggregate strategy employed by Zhou's group, Hikabe et al.[24] used mice embryonic ovarian somatic cells to create reconstituted ovaries with mPGCLC. By using cell culture protocols destined to mature primary follicles and mouse PGC into functional metaphase II oocytes,[25,26] the group obtained metaphase II oocytes that would later be fertilised via ICSI and produce healthy offspring, thus completing the full oogenesis *in vitro*.[24,27] However, this method, had a live birth rate 20 times lower compared with oocytes in vivo attributable to errors during the epigenetic reprogramming caused by the short *in vitro* culture time to perform the *in vitro* differentiation compared to the time it lasts *in vivo*.[1]

As previously described, the most recent methods for PGCLC maturation required the use of somatic cells, which makes the application of the protocol not appropriate for humans. Jung et al.[22] tried to solve this by reproducing ovarian follicles *in vitro* without human gonad somatic cells as a supporting structure (Table 1).[14,20-23] Starting hESC were differentiated into hPGC on top of fibroblast feeders cultured with BMP4 and BMP8A. Based on the knowledge that DAZL, a RNA-binding protein, regulates the transition from pluripotency of germ cells, this group overexpressed DAZL and BOULE in hPGCLC to induce meiosis *in vitro*. Once the expression of meiotic factors PRDM9 (expressed in the nucleus during preleptotene), H2AX (key histone for the remodelling of chromatin), and SYCP3 (which participates in the formation of the synaptonemal complex) was confirmed, the cells were transduced using human recombinant GDF4 and BMP15. After 9 days in culture, structures resembling follicles arose and the expression of ZP2 and NOBOX (oocyte markers) in the centre of the follicles was observed. Granulosa cells specific genes such as *CYP19A* and *RSP01* were also present. This was the first study that obtained a structure similar to an ovarian follicle that expressed both oocyte and granulosa cell factors.[22]

In summary, the *in vitro* differentiation of hPGCLC into functional gametes is still not completely successful since the functionality of the resulting cells is yet to be proven. A first approximation to this evaluation was performed by Yamashiro et al.[23] in 2018, who induced hPGCLC from a line of hiPSC and coaggregated them with somatic ovarian cells from 12-day-old mice embryos, thus creating xenogeneic reconstituted ovaries (Table 1).[14,20-23] After 27 days of culture, both female and male cells derived from hPGCLC expressed early germ cell factors TFAP2C and SOX17, as well as DAZL and DDX4 which determine that these had the potential to differentiate into gonocytes or oogonia. One month later, cells also expressed meiosis genes *SYCP3* and *REC8*. The resulting cells formed a follicle-like structure and, from Day 120 of culture onwards, they expressed *STRA8*, indicating their readiness for meiosis. The authors also proved that the gene expression profile of these cells was very similar to that of oogonia *in vivo*. Furthermore, derived cells were able to respond to retinoic acid in preparation for meiosis[23] and silenced male germline-specific

genes.[28] Finally, these cells progressively erased their paternal and maternal imprint; however, their reactivation of the inactive X chromosome was proven to be inefficient.[23] An additional summary of the most recent studies regarding the obtention of germ cells *in vitro* is shown in Table 1.[14,20-23]

Transdifferentiation

The birth of cellular reprogramming in 2006 allowed for the dedifferentiation of adult cells into pluripotent cells via the use of the Yamanaka factors.[29,30] These iPSC could be later cultured with lineage-specific factors and, theoretically, differentiate into any other cell types. Based on this approach of reprogramming terminally differentiated cells into a different cellular lineage, Medrano et al.[31] used a transdifferentiation strategy to obtain germ cells from somatic cells bypassing their pluripotent state. Starting from a foreskin fibroblast primary culture and a mesenchymal stem cell line, they overexpressed 6 key genes in the XY starting somatic cells: *PRDM1*, *PRDM14*, *LIN28*, *DAZL*, *VASA*, and *SYCP3*. The resulting cells exhibited an increase in expression of Fragilis and STELLA, markers for early germline differentiation, as well as early markers of PGC like *SOX17*. As the culture progressed, a decrease in the expression of premeiotic and early factors was observed, together with an increase in expression of late postmeiotic markers. However, the efficiency of the entire process was low: only 0.5–1.0% of the starting cells were able to complete meiosis. Once the epigenetics of these cells were analysed, the group observed that even though the starting cells were male (n=46, XY), the cells resulting from this genetic modification showed a significant loss of methylation in the paternal imprinting genes, while their methylation in the maternal imprinting genes increased, proving that in absence of a male gonad environment, the determination of female sex prevails. Finally, the group conducted a functional test in which *in vitro* obtained cells were able to colonise previously sterilised mouse testicles, even then complete spermatogenesis could not be completed because of interspecific differences between murine and human spermatogenesis.[31] Despite achieving a low yield of recovered cells, this study became a starting point for protocols of direct differentiation of germ cells from adult somatic cells, which eliminates the need to isolate or induce pluripotent cells in order to achieve gametes.

In 2019, Zhang et al.[32] reduced the number of transgenes required for the transdifferentiation of goat bone marrow stem cells (BMDSC) into spermatocyte precursor cells to 3 genes: *STRA8*, *BOULE*, and *DAZL*.[33] The resulting cells were able to initiate meiosis and increase their expression of premeiotic factors *STELLA* and *c-KIT* compared to nontransfected cells, as well as postmeiotic markers *PIWIL2* and *SCYP3*. Thus, the resulting cells were able to initiate meiosis, arrive to the point of recombination, and undergo epigenetic erasure of the imprinting genes. Nonetheless, they were unable to demonstrate that these cells completed meiosis to result in functional haploid spermatozoa.[32]

ALTERNATIVES TO THE USE OF ESCS OR IPSCS

Because of the inefficiency in the *in vitro* derivation of human gametes and the lack of evidence ensuring their safety and correct functionality, their use in a clinical setting for reproductive use is currently far from reality. Nevertheless, alternative stem cell sources exist to obtain germ cells for a more feasible short-term clinical application.

SSC reside within the immature testicular tissue, which can be cryopreserved in prepubertal males that will be subjected to a potentially gonadotoxic treatment such as oncologic patients as a strategy to preserve their fertility. Following this strategy in 2019, Fayomi et al.[34] reported the birth of the first nonhuman primate conceived using spermatozoa from rhesus macaque cryopreserved prepubertal testicular tissue which was later transplanted into the same castrated individual. In this study, one testicle was retrieved, cut into pieces, and cryopreserved. Immature tissue fragments were grafted under the skin from the same macaque's back and scrotum 7 months later. Once fragments were recovered and analysed 8–12 months post-grafting, seminiferous tubules exhibited complete spermatogenesis with multiple layers of VASA positive germ cells and acrosin positive post-meiotic spermatids. More than 70% of the seminiferous tubules contained elongated spermatogonia and/or spermatozoa. Spermatozoa were retrieved and

microinjected into oocytes, and the resulting embryos displayed a 67% arrival to blastocyst rate and 25% gestation rate, comparable to rates obtained through natural ejaculation. Eleven blastocysts were transferred to six receptors and one successful birth was achieved. Although this experiment was performed in castrated animals, and therefore requires validation in sterile individuals with intact testicles,[34,35] it introduces the application of the *in vivo* maturation technique of cryopreserved fragments as a theoretically viable option for fertility preservation of prepubertal males.[36]

However, there are limitations to introducing this and other cellular transplantation techniques into clinical practice. Firstly, the origin of the transplanted sample presents the risk of reintroducing malignant cancer cells from the same patient. Thus, the auto-transplant strategy would not be applicable in children with leukaemia, lymphoma, or testicular cancer unless the safety of the transplant is ensured.[34] Secondly, resumption of spermatogenesis in humans after reimplantation of immature testicular tissue has never been tried. Gonads are complex entities where cells require a specific environment to complete gametogenesis. Therefore, bioengineering approaches to this issue can be the creation of biocompatible scaffolds that mimic the testicle's or ovary's microenvironment to promote the differentiation of germinal stem cells into functional haploid gametes *in vitro*.[37]

Given the difficulty to retrieve gonadal somatic cells to act as a scaffold for PGC differentiation, several research groups looked for alternative sources of pluripotent stem cells to obtain germ cells. BMDSC can restore oogenesis by migrating and settling in the ovarian niche, facilitating follicular growth, neovascularisation, and proliferation of the ovarian stroma. This has been observed in mice[38] and human patients with premature ovarian failure caused bychemotherapy treatment who, after a bone marrow transplant, became pregnant naturally.[39] In fact, the ovarian infusion of autologous BMDSC in 17 low-responding women undergoing *in vitro* fertilisation cycles significantly increased the antral follicle count 2 weeks from the transplant, resulting in five live births.[40] However, this technique does not involve the creation of new follicles or oocytes but mainly the 'awakening' of existing ones.[41]

CURRENT CHALLENGES AND FUTURE PERSPECTIVES

In humans, hPGCLC have been successfully induced from hESC and hiPSC; however, differentiation of these cells into mature and functional gametes is still ineffective.[23] The available information about the development of the human germline is limited and focusses on the early stages of the differentiation process.

Besides its academic application, producing functional gametes *in vitro* would entail a substantial decrease in the shortage of ovum donors for fertility treatments and would offer a reproductive option to patients who are otherwise unable to have children.[27] With respect to the latter, the current survival rate for childhood cancer is 80% and rising, which calls for the development of strategies that allow them to pursue their reproductive wish during adulthood whenever cryopreservation of mature gametes is not possible.[1,37] For this scenario, in males, there are several experimental techniques based on the cryopreservation of immature testicular tissue that are being studied in animal models; spermatogonial stem cell transplantation,[35,42] testicular grafting, and *in vitro* spermatogenesis, which is currently nonviable with human cells since the process is arrested at the maturation stage of spermatogonia. The inefficiency of the latter is its greatest limitation, since most of the initial germ cells are lost during culture in the lab.[43,44]

Furthermore, before gametes derived from hESC or hiPSC could be used for reproductive purposes, the genome and epigenome of both the starting cells and the resulting gametes need to be assessed to check that mutations or epimutations are not passed on to the offspring.[6,45] It would be an irresponsibility for researchers to ignore the possibility of causing epigenetic errors that would result in aberrant embryogenesis or one that would entail unpredicted adverse effects in the offspring.[46] Furthermore, since the last phase of the process has not been successfully reproduced with human cells in the laboratory, there is no proof of concept that spermatozoa generated from *in vitro* derivation of pluripotent stem cells would be able to correctly pack chromatin, substitute histones for protamines, and activate oocytes. Evidence that the female counterpart

created using the same technique would be able to complete meiosis, repair DNA damage, disintegrate the nucleus of the spermatozoa, and form both pronuclei correctly would also be required.[47]

CONCLUSION

Overall, for now, the most promising use of *in vitro* gametogenesis is to serve as a study model, with a particular interest for researchers in basic biology, more than an alternative source of functional gametes.[1,48] Nevertheless, recent advances described in this review suggest a promising future with a rise of new reproductive therapies following optimisation of these strategies.

References

1. Nagamatsu G, Hayashi K. Stem cells, *in vitro* gametogenesis and male fertility. Reproduction. 2017;154(6):F79-91.
2. Sun YC et al. Reconstitution of gametogenesis in vitro: meiosis is the biggest obstacle. J Genet Genomics. 2014;41(3):87-95.
3. Tang WWW. Specification and epigenetic programming of the human germ line. Nat Rev Genet. 2016;17(10):585-600.
4. Chen D et al. The TFAP2C-regulated OCT4 naive enhancer is involved in human germline formation. Cell Rep. 2018;25(13):3591-602e5.
5. Kobayashi T, Surani MA. On the origin of the human germline. Development. 2018;145(16):dev150433.
6. Saitou M, Miyauchi H. Gametogenesis from pluripotent stem cells. Cell Stem Cell. 2016;18(6):721-35.
7. Ohinata Y et al. A signaling principle for the specification of the germ cell lineage in mice. Cell. 2009;137(3):571-84.
8. O'Brien MJ et al. A revised protocol for *in vitro* development of mouse oocytes from primordial follicles dramatically improves their developmental competence. Biol Reprod. 2003;68(5):1682-6.
9. Sato T et al. *In vitro* production of fertile sperm from murine spermatogonial stem cell lines. Nat Commun. 2011;2:472.
10. Hayashi K et al. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell. 2011;146(4):519-32.
11. Hackett JA et al. Tracing the transitions from pluripotency to germ cell fate with CRISPR screening. Nature Commun. 2018;9(1):4292.
12. Hayashi K et al. Offspring from oocytes derived from *in vitro* primordial germ cell-like cells in mice. Science. 2012;338(6109):971-5.
13. Irie N et al. SOX17 Is a critical specifier of human primordial germ cell fate. Cell. 2015;160(1-2):253-68.
14. Sasaki K et al. Robust *in vitro* induction of human germ cell fate from pluripotent stem cells. Cell Stem Cell. 2015;17(2):178-94.
15. Nakaki F et al. Induction of mouse germ-cell fate by transcription factors in vitro. Nature. 2013;501(7466):222-6.
16. Kobayashi T et al. Principles of early human development and germ cell program from conserved model systems. Nature. 2017;546(7658):416-20.
17. Murakami K et al. NANOG alone induces germ cells in primed epiblast *in vitro* by activation of enhancers. Nature. 2016;529(7586):403-7.
18. Fang F et al. A PAX5-OCT4-PRDM1 developmental switch specifies human primordial germ cells. Nat Cell Biol. 2018;20(6):655-65.
19. Zhou Q et al. Complete meiosis from embryonic stem cell-derived germ cells in vitro. Cell Stem Cell. 2016;18(3):330-40.
20. Ishikura Y et al. *In vitro* derivation and propagation of spermatogonial stem cell activity from mouse pluripotent stem cells. Cell Rep. 2016;17(10):2789-804.
21. Sosa E et al. Differentiation of primate primordial germ cell-like cells following transplantation into the adult gonadal niche. Nat Commun. 2018;9(1):5339.
22. Jung D et al. *In vitro* differentiation of human embryonic stem cells into ovarian follicle-like cells. Nat Commun. 2017;8:15680.
23. Yamashiro C et al. Generation of human oogonia from induced pluripotent stem cells in vitro. Science. 2018;362(6412):356-60.
24. Hikabe O et al. Reconstitution *in vitro* of the entire cycle of the mouse female germ line. Nature. 2016;539(7628):299-303.
25. Morohaku K et al. Complete *in vitro* generation of fertile oocytes from mouse primordial germ cells. Proc Natl Acad Sci U S A. 2016;113(32):9021-6.
26. Hayashi K, Saitou M. Perspectives of germ cell development *in vitro* in mammals. Anim Sci J. 2014;85(6):617-26.
27. Makar K, Sasaki K. Roadmap of germline development and *in vitro* gametogenesis from pluripotent stem cells. Andrology. 2019;00:1-10.
28. Nagaoka Si, Saitou M. Reconstitution of female germ cell fate determination and meiotic initiation in mammals. Cold Spring Harb Symp Quant Biol. 2017;82:213-22.
29. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663-76.
30. Takahashi K et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861-72.
31. Medrano JV et al. Human Somatic cells subjected to genetic induction with six germ line-related factors display meiotic germ cell-like features. Sci Rep. 2016;6:24956.
32. Zhang YL et al. Induction of goat bone marrow mesenchymal stem cells into putative male germ cells using mRNA for *STRA8*, *BOULE* and *DAZL*. Cytotechnology. 2019;71(2):563-572.
33. Li PZ et al. Overexpression ff *STRA8*, *BOULE*, and *DAZL* genes promotes goat bone marrow-derived mesenchymal stem cells *in vitro* transdifferentiation toward putative male germ cells. Reprod Sci. 2017;24(2):300-12.
34. Fayomi AP et al. Autologous grafting of cryopreserved prepubertal rhesus testis produces sperm and offspring. Science. 2019;363(6433):1314-9.
35. Pelzman DL et al. Progress in translational reproductive science: testicular tissue transplantation and *in vitro* spermatogenesis. Fertil Steril. 2020;113(3):500-9.

36. Focus on Reproduction. More developments in *in vitro* gametogenesis. 2019. Available at: https://www.focusonreproduction.eu/article/ESHRE-Meetings-More-developments-in-in-vitro-gametogenesis. Last accessed: 30 March 2019.
37. Giudice MG et al. Update on fertility restoration from prepubertal spermatogonial stem cells: how far are we from clinical practice? Stem Cell Res. 2017;21:171-7.
38. Martin JJ et al. Reconstitution of oogenesis in mice using bone marrow-derived stem cells aggregated with fetal ovarian somatic cells. Cell. 2019;8(2):93.
39. Herraiz S et al. Fertility rescue and ovarian follicle growth promotion by bone marrow stem cell infusion. Fertil Steril. 2018;109(5):908-18e2.
40. Herraiz S et al. Autologous stem cell ovarian transplantation to increase reproductive potential in patients who are poor responders. Fertil Steril. 2018;110(3):496-505.e1.
41. Bhartiya D et al. Making gametes from alternate sources of stem cells: past, present and future. Reprod Biol Endocrinol. 2017;15(1):89.
42. Tian J et al. Relative safety of various spermatogenic stem cell purification methods for application in spermatogenic stem cell transplantation. Stem Cell Res Ther. 2019;10(1):382.
43. Medrano JV et al. [Current state regarding fertility cryopreservation in pre-pubertal boys]. Rev Int Androl. 2018;18(1):27-34. (In Spanish).
44. Medrano JV et al. Basic and clinical approaches for fertility preservation and restoration in cancer patients. Trends Biotechnol. 2018;36(2):199-215.
45. Mathews DJ et al. Pluripotent stem cell-derived gametes: truth and (potential) consequences. Cell Stem Cell. 2009;5(1):11-4.
46. Ishii T, Saitou M. Promoting *in vitro* gametogenesis research with a social understanding. Trends Mol Med. 2017;23(11):985-8.
47. Handel Ma et al. Applying "gold standards" to in-vitro-derived germ cells. Cell. 2014;157(6):1257-61.
48. Ge W et al. Skin-derived stem cells as a source of primordial germ cell- and oocyte-like cells. Cell Death Dis. 2016;7(11):e2471.

Oocyte Cryopreservation in Emergency Situations: Perspectives and Reality

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Abstract

Increased incidence of global recorded cancer, unforeseen circumstances in assisted reproductive technology, a pandemic situation, and surgical interventions which can cause impairment of the reproductive system all necessitate urgent fertility preservation. Unfortunately, the application of successfully developed methods for oocyte and embryo cryopreservation is not possible in some situations because of contraindications for inducing superovulation, inability to delay other treatments, or in the case of prepubertal patients; in these cases, cryopreservation of ovarian tissue may be an alternative method. Despite current achievements in ovarian tissue low-temperature preservation, only 130 children have been born using this method. Further development of this technique and methods for *in vitro* maturation of immature oocytes, following their cryopreservation and use in assisted reproductive technology, as well as a differentiated approach for the selection of mature oocytes obtained without preliminary superovulation are needed. This review outlines the modern achievements and future prospects of female fertility preservation in emergency situations by cryopreservation of oocytes with different quality and maturity states.

INTRODUCTION

The need to preserve women's fertility urgently arises when unforeseen circumstances occur in assisted reproductive technology (ART), such as when there is no possibility to fertilise aspirated oocytes, in a pandemic situation, when there are high risks of complications during pregnancy for patients undergoing infertility treatment, following surgical interventions that can cause impairment of the reproductive system, and in cancer patients requiring immediate

chemotherapy with gonadotoxic effects.[1,2] Annually, there is a global increased incidence of cancer so development of methods for preservation of fertility in patients after successful cancer treatment is both relevant and important.[3] There are currently several fertility conservation strategies.[4] The first strategy is the 'gold standard' of emergency ovarian stimulation followed by embryo or oocyte cryopreservation. However, this method can be inappropriate for single women refusing sperm donation and for prepubertal girls.[5] Mature oocyte retrieval is associated with hormonal stimulation of superovulation, contraindicated in hormone-dependent tumours.[6] Moreover, ovarian stimulation protocol delays the initiation of cancer therapy which may increase the likelihood of relapse.[7] The second strategy is ovarian tissue extraction followed by ovarian tissue freezing and autotransplantation or *in vitro* maturation (IVM), and is currently considered experimental. The third strategy includes ovarian protection techniques such as oophoropexy but their efficacy is debatable. Further study and development of the experimental strategies is relevant, because they are an alternative for those strategies that cannot be applied for female fertility preservation.

To date, cryopreservation of ovarian tissue is under development and a little more than 130 children have been born following its use.[8] In addition, the use of autotransplantation in patients who have recovered after cancer may cause metastasis unless cases are carefully selected. Cryopreservation of individual follicles could solve this problem; however, after thawing it is necessary to carry out *in vitro* growth which has not yet led to the production of mature oocytes.[9]

The absence of preliminary ovarian stimulation means that retrieval of immature oocytes will require further IVM. There is no difference in the number of oocytes or their IVM rates relating to the phase of the menstrual cycle at which oocyte retrieval is performed, and IVM can be a promising tool for patients with breast cancer seeking urgent oocyte cryopreservation.[10] But, even in cases where retrieved oocytes are mature, they may have morphological abnormalities, resulting from intrinsic or extrinsic factors, that can impact on the cryopreservation efficacy and further fertilisation outcomes.[11] In this regard, the oocytes' maturity and their morphology can affect the efficacy of fertility preservation and ongoing fertilisation outcomes. The aim of this study was to evaluate the current options for female fertility preservation in emergency situations by cryopreservation of oocytes with different quality and maturity states.

METHODS

Literature searches were conducted in PubMed using the keywords "emergency fertility preservation", "fertility preservation for cancer patient", "oocyte vitrification", "oocyte *in vitro* maturation", and other words related to the current review. The studies were selected based on their relevance to the material presented in the review, the availability of the full text of the papers, as well as those that published the most recent data on the issue.

Oocyte Cryopreservation

In 2013, the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART) published a joint document, 'Mature Oocyte Cryopreservation: A Guideline', which addressed advances in techniques to freeze human eggs that have resulted in significant recent improvements in pregnancy success. Based on the current state of evidence, modern procedures to cryopreserve oocytes should no longer be considered experimental.[12] Since then, many more ART clinics around the world have actively started to offer oocyte cryopreservation as an effective method of preserving fertility.[13-16]

The fundamental science underpinning cryopreservation of female reproductive cells, including the basis for the two most frequently used techniques (controlled-rate slow-cooling or vitrification), is beyond the scope of this review and has been recently discussed.[17,18] The first pregnancy was achieved following oocyte cryopreservation using the controlled-rate slow freezing method in 1986.[19] After this, slow freezing has been considered the standard method for oocyte cryopreservation, and oocyte recovery of morphologically intact oocytes ranges from 50–60%. The decrease in oocyte survival was associated with the formation of ice crystals during freezing and thawing. Currently, avoiding the formation of ice crystals around or inside oocytes has led to improved oocyte cryopreservation by

the vitrification technique. The first pregnancy after oocyte vitrification was in 1999.[20] However, there are still a large number of scientific studies comparing the effectiveness of the slow-cooling method and vitrification.[21-23] Technological features of cryopreservation techniques do not determine the optimal method, since each has advantages and disadvantages (Box 1). However, different research findings and meta-analyses of oocyte survival rates, fertilisation outcomes, embryo development, and pregnancy rates have indicated that vitrification is a preferable method to slow freezing for mature oocytes (Table 1). These vitrification advantages can probably be explained by the enhanced preservation of the ultrastructural characteristics of oocytes. The data from transmission electron microscopy studies suggest that recovery of perivitelline spaces and mitochondria was more complete after vitrification than after slow freezing.[27] The presence of normal oocyte meiotic spindle was greater after vitrification than after slow freezing (93.5% versus 72.0%; p=0.0128).[28] Moreover, the gene expression profile of metaphase II (MII) oocytes is differentially affected by slow freezing and vitrification compared with non-cryopreserved MII oocytes.[29] Slow freezing was associated with downregulation of genes involved in chromosomal structure maintenance (*KIF2C* and *KIF3A*) and cell cycle regulation (*CHEK2* and *CDKN1B*) that may lead to a reduction in the oocyte developmental competence after thawing compared with the vitrification procedure. This is confirmed by other data claiming that oocytes' potential to be fertilised and to develop into high-quality blastocysts was similar to embryos from fresh oocytes in oocyte donation programmes.[30]

Another challenge in mature oocyte cryopreservation is the presence of the meiotic spindle which consists of tubulin polymers sensitive to temperature fluctuation, along which the chromosomes are arranged ready for fertilisation. During cryopreservation, these microtubules can be depolymerised and cause impairment of proper chromosome segregation after thawing and fertilisation, such as nondisjunction, premature separation of sister chromatids, or the recently discussed reverse segregation which increases incidence of aneuploidies and possibly decreases mitochondrial activity.[31] After thawing of vitrified oocytes, the meiotic spindle microtubules are able to repolymerise.[32] The optimal time for this recovery depends on the cryopreservation method, the initial morphological and functional state of the oocytes, and the patient's age.[33] It was shown that the meiotic spindle was visualised in only 35.7% of oocytes immediately after thawing. However, after 3 hours of incubation at 37 °C in a culture medium, the meiotic spindle was visualised in 57.4% of female gametes.[34] It has been shown that meiotic spindle morphology and embryo ploidy are normally retained after oocyte vitrification and do not suffer negative impacts from the cryogenic cooling.[35,36]

Box 1: A comparison of oocyte cryopreservation methods.

	Cryopreservation method	
	Slow-cooling	**Vitrification**
Advantages	- Low cryoprotectant concentration - Controlled temperature steps	- Not a time-consuming method; - Cost-effective
Disadvantages	- Expensive specialised equipment - Time-consuming method	- Cytotoxicity due to the high cryoprotectant concentration - Risk of cross-contamination between the samples in liquid nitrogen in an open system - Requires highly qualified specialists

Table 1: A comparison of study outcomes for oocyte cryopreservation methods.

	Cryopreservation method	
	Slow-cooling	**Vitrification**
Survival rate	66.1% (298/451) (RR = 1.23; 95% CI: 1.02-1.49; p<0.05); and three cohort studies (RR = 1.23; 95% CI: 1.11–1.36; p<0.0001) (Rienzi L et al.,[24] 2017)	82.3% (602/731) (RR: 1.23; 95% CI: 1.02–1.49; p<0.05); and three cohort studies (RR: 1.23; 95% CI: 1.11–1.36; p<0.0001) (Rienzi L et al.,[24] 2017)
Fertilisation rate	64.6% (Fadini R et al.,[25] 2009)	72.8% (Fadini R et al.,[25] 2009)
Blastocyst-formation rate	12.0% (Cao YX et al.,[26] 2009)	33.1% (Cao YX et al.,[26] 2009)
Clinical pregnancy rate	7.6% (Fadini R et al.,[25] 2009)	18.2% (Fadini R et al.,[25] 2009) Increased (RR: 3.86; 95% CI: 1.63–9.11; p=0.002); two RCT, 106 women, I^2 = 8%, moderate-quality evidence (Glujovsky D et al.,[23] 2014) Increased per cycle (RR: 2.81; 95% CI: 1.05–7.51; p=0.039); Increased per transfer (RR: 1.81; 95% CI: 0.71–4.67; p=0.214); Increased per cryopreserved oocyte (RR: 1.14; 95% CI: 1.02–1.28; p=0.018) (Rienzi L et al.,[24] 2017)
Implantation rate	4.3% (Fadini R et al.,[25] 2009)	9.3% (Fadini R et al.,[25] 2009)

CI: confidence interval; RCT: randomised, controlled trials; RR: relative risk.

Another point to consider is oocyte morphological deviations, which exist among oocytes and may result from intrinsic factors such as maternal age and genetic defects or extrinsic factors such as stimulation protocols, culture conditions, and nutrition. These oocyte morphological features may affect the developmental competence and implantation potential of the derived embryo.[11] They can also impact oocyte cryopreservation outcomes for individual cancer patients who wish to preserve their fertility. As with infertility services in general, counselling services for cancer patients who wish to engage with oocyte cryopreservation must be robust and informative, especially because decision making may need to progress quickly.

Should All Aspirated Mature Oocytes Be Cryopreserved?

According to the Istanbul consensus on the quality assessment of gametes and embryos, there are optimal morphological characteristics of oocytes: spherical structure; homogeneous zona pellucida (ZP); translucent, inclusions-free cytoplasm; and an adequate first polar body (PB) with no fragmentation sign.[37]

Oocyte structural abnormalities are divided into intra and extracytoplasmic. The first category includes different types and degrees of cytoplasmic granulations, variations in colour (dark-coloured cytoplasm, slightly diffused or excessive whole/centrally located granulation, lipofuscin bodies), the appearance of refractile bodies and smooth endoplasmic reticulum clusters, vacuolisation in the body of the oocyte, the maturity of the nucleus, and the presence of a normal meiotic spindle. The second category includes the presentation of the first PB (size, fragmentation) shape abnormalities (irregular shape of the MII oocyte), ZP abnormalities (dark or thick ZP), and perivitelline space abnormalities (large size, granularity). These morphological characteristics can be used as criteria for determining the quality of oocytes, and some may be predictors of their successful recovery after cryopreservation.[38] ZP anomalies can arise from impaired secretion and glycoprotein matrix formation.[39] Oocyte survival after cryopreservation depends on the morphological characteristics of ZP. The thickness can influence the cryoprotectant penetration; before cryopreservation, it is necessary to evaluate the morphological structure of the ZP and individually select the suitable time for cryoprotectant exposure, depending on the ZP thickness and oocyte osmotic reaction.[40]

The morphological structure of the first PB can be regarded as a reflection of the oocyte's post-vascular age. Oocytes with an oval PB and a smooth surface and without fragmentation develop into morphologically normal embryos with high implantation potential. The first-PB morphological abnormalities were associated with a decreased fertilisation rate, but did not show any correlation with embryo quality.[41,42] The presence of an enlarged first PB is related to worse rates of fertilisation, cleavage, and high-quality embryos. However, the identification of the first-PB fragmentation does not seem to interfere with the results of intracytoplasmic sperm injection, suggesting that oocyte selection based on PB fragmentation may not contribute to the identification of embryos with high developmental ability. It has been shown that not only the morphological characteristics of the first PB, but also the angle of its location with respect to the pronuclei that appear after fertilisation, are important in predicting the quality of embryos.[43] Oocyte first-PB morphology observed prior to vitrification can predict post-warming survival, and developing non-invasive identification of predictive markers for oocyte survival potential remains relevant.

Vacuoles are cytoplasmic inclusions filled with a fluid identical to the fluid of the perivitelline space. They are considered a morphological feature of the degenerative process in the oocyte because they can interfere with further fragmentation or division of the embryo *in vitro*, which in turn inhibits the rate of successful blastulation.[44] The presence of endoplasmic reticulum aggregation is associated with a lower chance of successful pregnancy and, even if normal oocytes are transferred from one pool, the pregnancy rate decreases.[45] Despite the normal pregnancy rates obtained with oocytes with granular cytoplasm, >50% of pregnancies resulted in miscarriages and the implantation rate was only 5%.[46] Variation in clustering and distribution of mitochondria can reflect developmental competence, and mitochondrial assessment can be used for analysis in vitrified or warmed oocytes.[47]

In addition to morphological features, oocytes can differ in their physical and chemical properties which can be observed when the suspending-medium osmolarity changes during cryopreservation processes. An individual osmotic response of oocytes may be considered flexible within the established range during the oocytes' exposure to low concentrations of cryoprotectants and to vitrification solutions. This approach does not contradict the recommendations of other investigations. The 'weaker' oocytes require a shorter equilibration time in cryoprotectant solution, whereas 'stronger' oocytes require prolonged exposure to the same solution for effective loading.[48] Application of a flexible strategy helps to eliminate the risk of detrimental expansion or prolonged dehydration of cells, and the toxicological effects of cryopreservation media. This osmotic response during the equilibration procedure can be taken as an indicator of the oocyte's viability and may improve prediction of the cryopreservation outcomes; using a flexible time of exposure to cryoprotectant could increase the oocytes' survival.

Development of criteria for assessment of mature oocytes is very important for predicting the outcomes of cryopreservation and possible individualisation of cryopreservation protocols, which are important when performing oocyte cryopreservation in emergency situations.

'State of the Art' for Immature Oocyte Cryopreservation

Obtaining immature oocytes in emergencies can be possible, even in situations where it may be necessary to eliminate costly drugs and frequent monitoring as used in routine ovarian stimulation; to complete treatment within 2–10 days, while avoiding the use of hormones in patients with hormone-sensitive tumours; and to retrieve oocytes at any point in the menstrual cycle, even in the luteal phase. In addition, immature oocytes can also be collected from extracorporeal ovarian biopsy specimens or ovaries during caesarean section.[49] Emergency fertility preservation increases the number of cases of immature oocyte collection needing IVM. IVM programmes are already offered to poor responders or patients with polycystic ovary syndrome to avoid the risk of developing ovarian hyperstimulation syndrome caused by exogenous gonadotrophin stimulation.[50] IVM protocols involve the addition of specific molecules to the *in vitro* oocyte culture media which have been implicated in reversing this meiosis-arresting action including cyclic adenosine monophosphate and purines.[51,52] More than 5,000 babies have been born using IVM worldwide.[53] The first baby from a vitrified IVM oocyte was reported in 2009.[54] It is important not only that nuclear maturation progresses normally (reaching Stage MII), but that cytoplasmic (metabolic and structural changes in ooplasm) maturation also proceeds in step during IVM. Mammalian germinal vesicle (GV) stage immature oocytes can be divided into several types: nonsurrounded nucleolus (NSN); surrounded nucleolus (SN); partly NSN (pNSN) and SN (pSN); prematurely condensed NSN, pNSN, and pSN; and early diakinesis patterns. Maturation and embryo culture suggest that SN and pSN oocytes were more competent after fertilisation than NSN and pNSN oocytes; prematurely condensed pSN oocytes were more competent than prematurely condensed NSN/pNSN oocytes.[55]

One of the problems for the cryopreservation of immature oocytes is that oocytes are surrounded by a layer of cumulus cells which are essential participants in providing oocyte maturation and are sensitive to cryopreservation.[56] Currently, the cryopreservation protocols for the oocyte–crown–cumulus complex have not been successfully developed, meaning that oocytes must be released from these surrounding cells before cryopreservation. In this case, the connection between the oocyte and granulosa cells is disrupted which makes subsequent IVM more difficult. The outcomes of oocyte maturation and their subsequent fertilisation are dependent on the method of maturation. It was shown that supplementing growth hormone to the GV oocyte can lead, during *in vitro* culture, to an approximate 70.0% maturation rate. The fertilisation rate and rate of development to blastocyst were 73.1% and 25.0%, respectively.[57] These data confirm the promising use of GV oocytes for patient fertility preservation, which will be helpful in any requirement for emergency fertility preservation.

The problem of cryopreservation of immature oocytes raises the reasonable question of the best time to carry out cryopreservation: prior to or after *in vitro* maturation. However, several studies argue that the maturation rate was higher after vitrification at the GV stage.[58] In contrast, other studies have shown that the maturation and fertilisation rates are significantly higher in the group of oocytes that were matured before cryopreservation.[59-62] These results may be seen as paradoxical because mature oocytes at the MII stage have a meiotic spindle, which is extremely sensitive to temperature fluctuations and even insignificant fluctuations may cause depolymerisation of spindle microtubules, leading to chromosome segregation impairment, embryo aneuploidy, and developmental abnormalities. Moreover, vitrification can affect the GV oocyte ultrastructure; it was noted that there were significant changes in the structure of meiotic spindle of oocytes matured *in vitro* after vitrification.[62,63] The current leading strategy for fertility preservation using the cryopreservation of immature oocytes should be development of gamete-maturation conditions that first allow for the obtainment of good-quality MII oocytes, followed by subsequent cryopreservation, with vitrification currently seen to be the most promising protocol.

FUTURE RESEARCH

Large, randomised, controlled trials are required to compare the various approaches for female fertility preservation to ensure the selection of an effective protocol. Currently, clinical trials databases have registered and tested trials comparing types of vitrification systems (open and closed) for oocytes and therapeutic intervention outcomes (primed either with recombinant human chorionic gonadotropin or a gonadotropin-releasing hormone agonist) prior to oocyte retrieval for IVM in patients with breast cancer. There have also been studies of outcomes for ovarian tissue cryopreservation following IVM or autologous transplantation of a retrieved oocyte. Further studies for IVM administration and the development of effective methods for ovarian tissue cryopreservation are still required.

CONCLUSIONS

Although oocyte cryopreservation provides many benefits for fertility preservation in general, its importance for emergency fertility preservation has not been widely discussed. Experience in the cryopreservation of mature oocytes has been gained in elective practices such as IVF; however, it is necessary to develop evaluation criteria to predict the effectiveness of oocyte cryopreservation and application of individualised oocyte cryopreservation protocols. Using immature oocytes for *in vitro* maturation and cryopreservation is promising for patients requiring emergency fertility preservation; however, further studies of this process, and development of enhanced maturation and vitrification techniques, are necessary. In addition, the results at one clinic of the 15th year cryopreservation of oocytes for patients with cancer showed that only 4.5% patients returned to use their gametes.[64] In the future, cryopreservation of ovarian tissues or isolated ovarian follicles may also contribute significantly to fertility preservation in emergency situations. Nevertheless, offering fertility preservation is no longer considered optional and must be included in every therapeutic programme for women who receive an oncological diagnosis in their reproductive age. This in turn will require development of close collaborations between cancer and fertility centres to provide a holistic, patient-centered fertility preservation strategy for female oncological patients.

References

1. Pampanini V et al. Impact of first-line cancer treatment on the follicle quality in cryopreserved ovarian samples from girls and young women. Hum Reprod. 2019;34(9):1674-85.
2. Tesarik J. After corona: there is life after the pandemic. Reprod Biomed Online. 2020;40(6):760-2.
3. Ferlay J et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer. 2018;103:356-87.
4. Salama M, Mallmann P. Emergency fertility preservation for female patients with cancer: clinical perspectives. Anticancer Res. 2015;35(6):3117-27.
5. Fasano G et al. Outcomes of immature oocytes collected from ovarian tissue for cryopreservation in adult and prepubertal patients. Reprod Biomed Online. 2017;34(6):575-82.
6. Muñoz E et al. Ovarian stimulation in patients with breast cancer. Ecancermedicalscience. 2015;9:504.
7. Moravek MB et al. Long-term outcomes in cancer patients who did or did not pursue fertility preservation. Fertil Steril. 2018;109(2):349-55.
8. Leonel ECR et al. Cryopreservation of human ovarian tissue: a review. Transfus Med Hemother. 2019;46(3):173-81.
9. McLaughlin M et al. Metaphase II oocytes from human unilaminar follicles grown in a multi-step culture system. Mol Hum Reprod. 2018;24(3):135-42.
10. Grynberg M et al. Similar *in vitro* maturation rates of oocytes retrieved during the follicular or luteal phase offer flexible options for urgent fertility preservation in breast cancer patients. Hum Reprod. 2016;31(3):623-9.
11. Balaban B, Urman B. Effect of oocyte morphology on embryo development and implantation. Reprod Biomed Online. 2006;12(5):608-15.
12. Noyes N et al. Oocyte cryopreservation: is it time to remove its experimental label? J Assist Reprod Genet. 2010;27(2-3):69-74.
13. Cobo A et al. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril. 2008;89:1657-64.
14. Cobo A et al. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. Hum Reprod. 2010;25(9):2239-46.
15. Rienzi L et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod. 2010;25(1):66-73.
16. Parmegiani L et al. Efficiency of aseptic open vitrification and hermetical cryostorage of human oocytes. Reprod Biomed Online. 2011;23(4):505-12.
17. Yurchuk T et al. Science of cryopreservation in reproductive medicine - embryos and oocytes as exemplars. Early Hum Dev.

2018;126:6-9.

18. Rienzi LF et al. Perspectives in gamete and embryo cryopreservation. Semin Reprod Med. 2018;36(5):253-64.
19. Chen C. Pregnancy after human oocyte cryopreservation. Lancet. 1986;1(8486):884--6.
20. Kuleshova L et al. Birth following vitrification of a small number of human oocytes: case report. Hum Reprod. 1999;14(12):3077-9.
21. Levi-Setti PE et al. Evolution of human oocyte cryopreservation: slow freezing versus vitrification. Curr Opin Endocrinol Diabetes Obes. 2016;23(6) 445-50.
22. Youm HS et al. Vitrfication and slow freezing for cryopreservation of germinal vesicle-stage human oocytes: a Bayesian meta-analysis. Cryo Letters. 2017;38(6):455-62.
23. Glujovsky D et al. Vitrification versus slow freezing for women undergoing oocyte cryopreservation. Cochrane Database Syst Rev. 2014;(9):CD010047.
24. Rienzi L et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update. 2017;23(2):139-55.
25. Fadini R et al. Human oocyte cryopreservation: comparison between slow and ultrarapid methods. Reprod Biomed Online. 2009;19(2):171-80. Erratum in: Reprod Biomed Online. 2009;19(5):757.
26. Cao YX et al. Comparison of survival and embryonic development in human oocytes cryopreserved by slow-freezing and vitrification. Fertil Steril. 2009;92(4):1306-11.
27. Li YZ et al. Vitrification versus slow freezing of human oocytes: effects on ultrastructure and developmental potential. Reprod Dev Med. 2018;2(3);129-36.
28. Martínez-Burgos M et al. Vitrification versus slow freezing of oocytes: effects on morphologic appearance, meiotic spindle configuration, and DNA damage. Fertil Steril. 2011;95(1):374-7.
29. Monzo C et al. Slow freezing and vitrification differentially modify the gene expression profile of human metaphase II oocytes. Hum Reprod. 2012;27(7):2160-8.
30. García JI et al. Efficacy of oocyte vitrification combined with blastocyst stage transfer in an egg donation program. Hum Reprod. 2011;26(4):782-90.
31. Cimadomo D et al. Impact of maternal age on oocyte and embryo competence. Front Endocrinol (Lausanne). 2018;9:327.
32. Tamura AN et al. Impact of vitrification on the meiotic spindle and components of the microtubule–organizing center in mouse mature oocytes. Biol Reprod. 2013;89(5):112.
33. Asgari V et al. Time dependent effect of post warming interval on microtubule organization, meiotic status, and parthenogenetic activation of vitrified *in vitro* matured sheep oocytes. Theriogenology. 2011;75(5):904-10.
34. Rienzi L et al. Polscope analysis of meiotic spindle changes in living metaphase II human oocytes during the freezing and thawing procedures. Hum Reprod. 2004;19(3):655-9.
35. Buderatska N et al. Does human oocyte cryopreservation effect equally on embryo chromosome aneuploidy? Cryobiology. 2020;93:33-6.
36. De Munck N et al. Chromosomal meiotic segregation, embryonic developmental kinetics and DNA (hydroxy)methylation analysis consolidate the safety of human oocyte vitrification. Mol Hum Reprod. 2015;21(6):535-44.
37. Alpha scientists in reproductive medicine and ESHRE special interest group of embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011;26(6):1270-83.
38. Rienzi L et al. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. Hum Reprod Update. 2010;17(1):34-45.
39. Choi JK et al. The crucial role of zona pellucida in cryopreservation of oocytes by vitrification. Cryobiology. 2015; 71(2):350-5.
40. Petrushko MP et al. Individualization of the cryopreservation procedure of biopsied embryos allows to increase their survival. Cryobiology. 2018;85:181-82.
41. Rienzi L et al. Significance of metaphase II human oocyte morphology on ICSI outcome. Fertil Steril. 2008;90(5):1692-700.
42. Navarro PA et al. Relationship between first polar body morphology before intracytoplasmic sperm injection and fertilization rate, cleavage rate, and embryo quality. Int J Gynaecol Obstet. 2009;104(3):226-9.
43. Setti AS et al. Oocyte morphology does not affect post-warming survival rate in an egg-cryobanking donation program. J Assist Reprod Genet. 2011;28(12):1177-81.
44. Petrushko M et al. Cryopreservation of incomplete compacted morulae and preliminary biopsy of excluded fragments. Zygote. 2019;27(6):386-91.
45. Otsuki J et al. The relationship between pregnancy outcome and smooth endoplasmic reticulum clusters in MII human oocytes. Hum Reprod. 2004;19(7):1591-7.
46. Kahraman S et al. Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. Hum Reprod. 2000;15(11):2390-3.
47. Nohales-Córcoles M et al. Impact of vitrification on the mitochondrial activity and redox homeostasis of human oocyte. Hum Reprod. 2016;31(8):1850-8.
48. Son WY et al. Immature oocyte for fertility preservation. Front Endocrinol (Lausanne). 2019;10:464.
49. Cha KY et al. Obstetric outcome of patients with polycystic ovary syndrome treated by *in vitro* maturation and *in vitro* fertilization-embryo transfer. Fertil Steril. 2005;83(5):1461-5.
50. Eppig JJ et al. Chemical signals that regulate mammalian oocyte maturation. Biol Reprod 1984;30(1):1-11.
51. Downs SM et al. Hypoxanthine is the principal inhibitor of murine oocyte maturation in a low molecular weight fraction of porcine follicular fluid. Proc Natl Acad Sci U S A. 1985;82(2):454-8.
52. Sauerbrun-Cutler MT et al. *In vitro* maturation and its role in clinical assisted reproductive technology. Obstet Gynecol Surv. 2015;70(1):45-57.
53. Chian RC et al. Live birth after vitrification of *in vitro* matured human oocytes. Fertil Steril. 2009;91(2):372-6.
54. Pan LZ et al. A new classification of the germinal vesicle chromatin configurations in pig oocytes. Biol Reprod. 2018;99(6):1149-58.
55. Li Y et al. Growth hormone promotes *in vitro* maturation of human oocytes. Front Endocrinol (Lausanne). 2019;10:485.
56. Ruppert-Lingham CJ et al. Membrane integrity and development of immature murine cumulus-oocyte complexes following slow cooling to -60 degrees C: the effect of immediate rewarming, plunging into LN2 and two-controlled-rate-stage cooling. Cryobiology. 2006;52(2):219-27.
57. Molina I et al. Osmotic-shock produced by vitrification solutions improves immature human oocytes *in vitro* maturation. Reprod Biol Endocrinol. 2016;14(1):27.
58. Fasano G et al. *In-vitro* maturation of human oocytes: before or after vitrification? J Assist Reprod Genet. 2012;29(6):507-12.

59. Lee JA et al. *In-vitro* maturation of germinal vesicle and metaphase I eggs prior to cryopreservation optimizes reproductive potential in patients undergoing fertility preservation. Curr Opin Obstet Gynecol. 2014;26(3):168-73.
60. Lee JA et al. Optimizing human oocyte cryopreservation for fertility preservation patients: should we mature then freeze or freeze then mature? Fertil Steril. 2013;99(5):1356-62.
61. Cao Y et al. Cryopreservation of immature and *in-vitro* matured human oocytes by vitrification. Reprod Biomed Online. 2009;19(3):369-73.
62. Liu MH et al. Ultrastructural changes and methylation of human oocytes vitrified at the germinal vesicle stage and matured *in vitro* after thawing. Gynecol Obstet Invest. 2017;82(3):252-61.
63. Shahedi A et al. The effect of vitrification on ultrastructure of human *in vitro* matured germinal vesicle oocytes. Eur J Obstet Gynecol Reprod Biol. 2013;167(1):69-75.
64. Specchia C et al. Oocyte cryopreservation in oncological patients: eighteen years experience of a tertiary care referral center. Front Endocrinol (Lausanne). 2019;10:600.

Premature Ovarian Insufficiency: A Review

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Abstract

Premature ovarian insufficiency is waning of ovarian function before the age of 40 years. This hypoestrogenic state is characterised by menstrual irregularities and loss of fertility in the patient. This review narrates evaluation, consequences, and management of this complex entity. Truncation in ovarian physiology at such an early age renders the patient prone for various short- and long-term health consequences which negatively affect physical and psychological well-being of the patients. Therefore, this review emphasises that timely initiation of hormonal therapy is mandatory to mitigate the distressing menopausal and/or other hypoestrogenic symptoms to improve the quality of life of such patients. Although much has been said about premature ovarian insufficiency, many aspects of this condition still need to be explored in order to identify this population subgroup before happening of the catastrophic event and to formulate strategies and interventions to delay the premature cessation of ovarian functions.

INTRODUCTION

Premature ovarian insufficiency (POI) is a hypergonadotropic hypogonadism condition which is characterised by impairment of ovarian function on a continuum before the age of 40 years. This age limit of 40 years has been taken as the cut-off age for POI as this age is approximately two standard deviations below the natural age of menopause. However, in contrast to menopause, complete cessation of follicular function does not occur in POI and intermittent ovulation can still occur. Therefore, the term 'premature ovarian insufficiency' is more accepted and scientifically correct than 'ovarian failure'. The incidence of POI is 1.0% by the age of 40 years, 0.1% by 30 years, and 1:10,000 by 20 years of age.[1]

POI is either spontaneous or iatrogenic in nature (surgery/radiotherapy/chemotherapy). Patients with ovarian insufficiency present either with complaint of menstrual irregularities or with hypoestrogenic symptoms. POI is the cause in 10–28% of women with primary amenorrhea and in 4–18% of women with secondary amenorrhea.[2,3] Psychological impact of POI is much more than its physical counterpart and needs to be addressed while managing such patients.

Although extensive literature is available about this entity, this review comprehensively encompasses the concept of POI along with its

cause, consequences, and available management options with the goal of providing relevant and current information to the healthcare provider of such patients.

METHOD

Literature was searched using the keywords 'premature ovarian insufficiency', 'premature ovarian failure', 'premature menopause', and other words related to the current review. The authors did not conduct an extensive search in order to find all the papers of interest acknowledging the availability of the vast literature on the topic. Studies were selected based on their relevance to the narration in the review.

PATHOPHYSIOLOGY

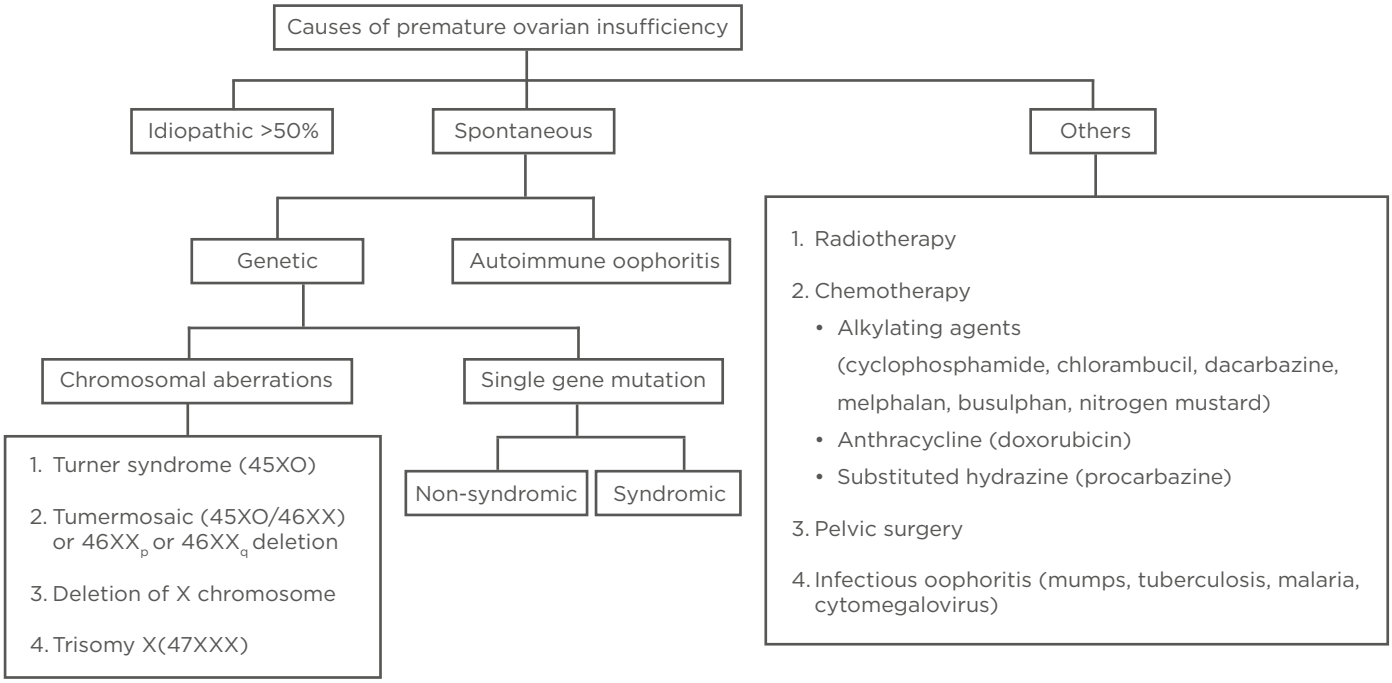
The number of primordial follicle peaks at 6–7 million by 20 weeks of gestation in a human female fetus. Thereafter, the follicles rapidly undergo atresia, leaving 1–2 million oocytes at birth and only 400–500 of these are ovulated before physiological menopause occurs.[4] This follicular atresia is regulated by interplay of various molecules which either stimulate the atretic process (androgens, TNF-alpha, and Fas ligand) or diminish it (oestrogen, gonadotropins, and nitric oxide). Thus, attrition of follicles in POI can occur by any of the three mechanisms: a smaller number of follicles from the beginning, faster atretic rate, or impaired recruitment of the follicles.[5]

AETIOLOGY

The various causes of POI are summarised in Figure 1. Despite research carried out over decades to identify the cause of POI, more than half of the cases still remain idiopathic. Around 20–25% of cases have a genetic cause, out of which approximately 9% of cases are due to aberrations in the X chromosome.[6] Turner syndrome is the most common chromosomal aberration abnormality in patients with POI. These patients present with primary amenorrhea and lack of breast development, while Turner mosaic patients may menstruate spontaneously. Amongst the other genetic causes, fragile X premutation is the most well studied and occurs due to change in *FMR1* (Xq27.3). In this premutation, the number of CGG trinucleotide repeats increases and ranges from 55 to 200.[7] Affected individuals are at increased risk of POI, from 3% in sporadic cases to 15% if a family history of POI is present.[1] Any deletion or rearrangement near X inactivation centre (located at Xq13) leads to mild features in the patients which are similar to patients with Turner syndrome. Another gene located on the short arm of X chromosome which can lead to POI is *BMP15*.[8] The protein it produces plays an important role in the maturation of oocytes. Some autosomal genes have also been identified, the mutations of which lead to decreased ovarian activity (*FSHR/ LHCGR*).[9]

Various syndromes associated with POI are also enumerated in Figure 1. If damage occurs during early gonadal differentiation, it leads to the most severe form of gonadal dysgenesis. For example, in Denys Drash Syndrome, which is caused by *WT1* mutation, and adrenal failure caused by *SF1* mutation.[5] Another important cause of POI is ovarian autoimmunity. It is diagnosed when anti-ovarian antibodies are present along with histological evidence of lymphocytic oophoritis and presence of other autoimmune disorders.[10] Steroid cell antibodies (SCA) target the enzyme 17-hydroxylase and cytochrome p450 side-chain cleavage enzyme and destroy steroid-producing cells in the adrenal cortex, testis (Leydig cells), ovaries (theca cells), and placenta (syncytiotrophoblast) leading to autoimmune poly-endocrine syndrome type 1 and 2, Addison's disease, and POI. SCA are detected in 60–87% of patients with POI-associated adrenal involvement.[11] However, the major limitation in diagnosing autoimmune involvement is the high false-positive rate and poor specificity of these antibodies.[12]

Infectious oophoritis (viral/bacterial) has been considered as one of the causes of POI.[13] Iatrogenic causes include surgical oophorectomy/ radiotherapy/chemotherapy. Complete cessation of ovarian activity occurs at cumulative radiotherapy dose of 20 Gy in women <40 years and at 5 Gy in older women.[14] Chemotherapy with alkylating agents, anthracycline, and substituted hydrazine increases the risk of POI by a factor of 9, more so in teenagers and young adults.[15]



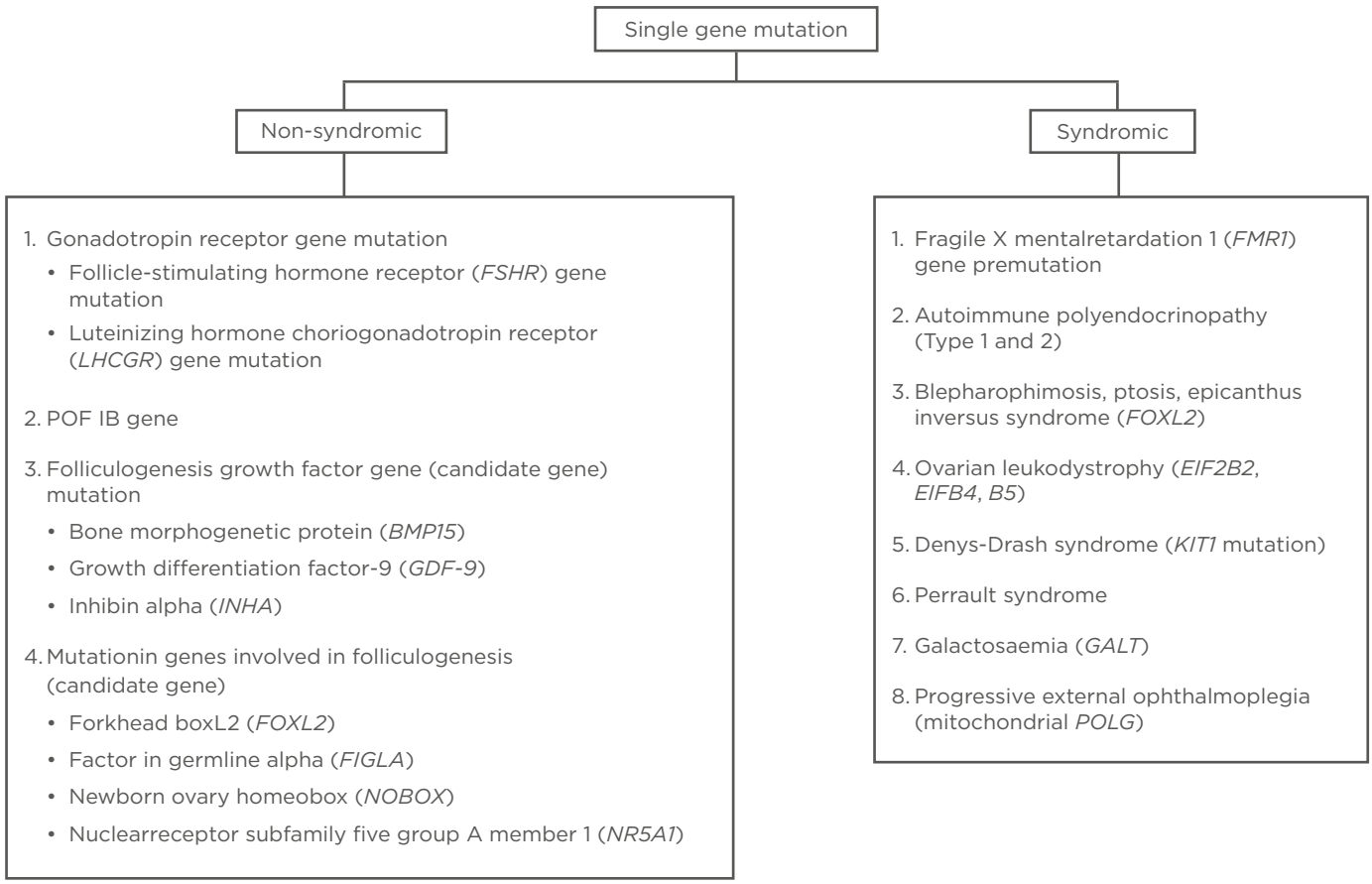


Figure 1: Summary of causes of premature ovarian insufficiency.

Box 1: Clinical history and examination of patients with premature ovarian insufficiency.

History	Examination
1. Age at menarche	1. Height of the patient
2. Maternal age at menopause	2. Weight of the patient
3. Frequency and duration of previous menstrual cycles	3. Secondary sexual characteristics
4. History of tobacco use/smoking	4. Skin pigmentation- vitiligo (autoimmune)/ hyperpigmentation (autoimmune adrenal insufficiency)
5. History of surgery/exposure to gonadotoxic substances	5. Dysmorphic features (to rule out other syndromic associations). Ophthalmologic examination (to look for blepharophimosis/ptosis)
6. Family history of premature ovarian insufficiency in first and second degree relative	6. Neck swelling (to rule out goitre [autoimmune aetiology])
7. History of mental retardation in any male family member	7. Local genital examination (to look for atrophic changes)
8. History of any genetic disorders in the family	
9. History of autoimmune disorder in self/family	

CLINICAL ASSESSMENT

Women with POI should be asked about personal, menstrual, medical, and family history in detail. The history that needs to be asked in the questionnaire and examination points are described in Box 1. Positive family history is present in up to 30% of POI cases; therefore, detailed family history of POI should be sought.[16] These patients should be asked about clinical symptoms which can be consequent to either POI alone or to associated autoimmune/genetic syndromes. Patients with POI experience menopausal symptoms such as vasomotor symptoms (commonest),[17] altered mood, tiredness, vaginal dryness, dyspareunia, urinary frequency, incontinence, and reduced libido. Around 12–14% of patients do not experience any menopausal symptoms,[18] especially patients in whom POI occurred before menarche because these symptoms are largely due to oestrogen withdrawal, and not deficiency.

INVESTIGATIONS

Diagnostic work-up of such patients should include confirmation of the diagnosis and the ruling out of other comorbid conditions. Although different levels of follicle-stimulating hormone (FSH) have been taken as cut-off value, persistent rise of serum FSH level >30 IU/L on two different occasions, at least 4–6 weeks apart, confirms the diagnosis of POI in the setting of menstrual irregularities.[19-23] Other common causes of amenorrhea should be excluded, such as pregnancy, hyperprolactinemia, chronic medical illness, and polycystic ovarian syndrome. Investigations that should be performed in all the cases are summarised in Box 2. A progesterone withdrawal test should not be completed as it gives a false sense of assurance thus delaying the diagnosis.[24]

Once the diagnosis of POI is confirmed, one should look for the cause of this condition. Chromosomal analysis, fragile X premutation testing, screening for thyroid antibody, and the measuring of 21-hydroxylase antibody levels can be done to confirm aetiology in patients with POI, wherever needed.[25-27] Autosomal gene mutation testing and anti-ovarian antibody testing is not routinely recommended. Ovarian biopsy is the gold standard test to diagnose ovarian autoimmune disorder. However, being an invasive test, it is not performed routinely. A bone density scan (DEXA scan) should be done to measure baseline bone mineral density (BMD) and if the bone density is in the normal range and the patient is compliant on hormone replacement therapy (HRT), this test can be repeated every 2–3 years.[18]

Box 2: Investigations to be done in all cases of premature ovarian insufficiency.

Definite tests	Rationale
1. Human chorionic gonadotropins	To rule out other causes of amenorrhea
2. Follicle-stimulating hormone (two samples at least 4–6 weeks apart)	
3. Thyroid-stimulating hormone	
4. Serum prolactin	
5. Antithyroid antibody (thyroid peroxidase antibody)	To look for associated autoimmune disorders
6. Anti 21-hydroxylase antibody by immunoprecipitation test	
7. Karyotype and *FMR1* premutation analysis	To look for genetic aetiology
8. Serum anti-müllerian hormone	To assess ovarian reserve
9. Dual-energy X-ray absorptiometry scan	To measure bone mineral density
10. Vitamin D status	To assess baseline levels
11. Ultrasound pelvis for endometrial thickness, ovarian volume, and antral follicle count (oestrogen status)	To assess oestrogen status and ovarian reserve

Tests with Poor Predictability

1. Progesterone withdrawal test
2. Serum oestradiol level
3. Ovarian antibody screening
4. Morning serum cortisol

LONG-TERM CONSEQUENCES

Premature withdrawal of oestrogen leads to premature ageing of various organ systems which are a primary target of oestrogen action and this may manifest as various diseases/disorders in the body. These effects are summarised above.

Cardiovascular System

Cardiovascular diseases are the main culprit behind shortened life expectancy of patients with untreated POI. A significant association was found between cardiovascular disease and early menopause in a meta-analysis.[28] Rocca et al.[29] showed that mortality was significantly increased in women who had a bilateral oophorectomy before the age of 45 years compared to those women who did not have an oophorectomy. In another study by Gordon et al.,[30] postmenopausal women in their fourth decade were found to have increased incidence of cardiovascular disease compared to premenopausal age-matched controls.

Bone Health

Lack of oestrogen increases bone remodelling, but bone resorption exceeds bone formation. The net effect is bone loss and in addition to that, slow mineralisation of new bone leads to low BMD. Almost 50% of affected women have significantly reduced BMD within 18 months of diagnosis and 2/3 have low BMD levels which put them at a high risk of hip fractures.[31,32] After 2–9 years of the diagnosis, there is a reduction of 2–3% in BMD at spine and hip.[18]

Effect on Cognition

Women with POI are at increased risk of cognitive impairment. Increased risk of cognitive impairment or dementia has been seen in women who undergo oophorectomy (unilateral/bilateral) before the onset of menopause.[33] Bove et al.[34] reported that premature surgical menopause is associated with faster decline in global cognition and memory. It was also studied that a HRT duration of at least 10 years (if started within 5 years of perimenopausal period) had protective effect on global cognition.

Genital Organ Dysfunction

Women with POI might have poor sexual performance owing to pain and vaginal dryness because of the impact of a lack of oestrogen. Androgen deficiency is the cause of low libido, poor genital arousal, and orgasm, as well as of blunted motivation and diminished wellbeing.[35,36] The loss of follicular function will result in infertility.

Psychological Effect

Sudden diagnosis of loss of fertility and menstruation can be unexpected and women often express anxiety, depression, or anger that is usually underestimated by the clinicians. It can be associated with increased lifetime risk of major depression and anxiety.[37,38] Women with POI have lower perceived social support and self esteem, which in turn affects their quality of life.[39,40]

MANAGEMENT

POI should ideally be managed by a multidisciplinary team comprising a gynaecologist, an endocrinologist, and a psychologist. The treatment of POI has the following components:

- HRT.
- Fertility regulation.
- Psychological support.

Hormone Replacement Therapy

Replacement of oestrogen is the first-line treatment of POI with the aim of relieving menopausal symptoms and improving cardiovascular, sexual, and bone health. It can induce secondary sexual characteristics in adolescents with POI. If breast development is incomplete or absent, oestrogen therapy should be initiated in low doses and should be slowly increased before starting progesterone to avoid tubular breast formation.[23]

Benefits of Hormone Replacement Therapy

HRT improves lipid profile by increasing serum high-density lipoprotein and decreasing total cholesterol and low-density lipoprotein. Overall, there is a 24% reduction in the risk of coronary artery disease after starting HRT.[41] It improves BMD and thus significantly reduces risk of fracture.[42] It also reverses the urogenital atrophy caused by oestrogen deprivation as well as relieves vasomotor symptoms. Thus, it improves the overall quality of life of the patient.

Routes of Administration

Oestrogen can be given orally or by a transdermal route. The transdermal route is preferred as the first pass effect by the liver is eliminated, thus lowering the risk of venous thromboembolism (VTE).[43] A study by Langrish et al.[44] showed better cardiovascular health outcomes amongst women with POI who received transdermal oestradiol and norethisterone. Oestrogen can be given as either 1–2 mg oral 17-β-oestradiol daily, 100 µg transdermal oestradiol, or 0.625–1.250 mg conjugated equine oestrogen. Progesterone can be administered by oral/transdermal/uterine route to prevent endometrial hyperplasia that can result from unopposed action of oestrogen in patients with an intact uterus (continuously/sequentially). Women can choose to have a levonorgestrel intrauterine device if they want effective contraception also. HRT has to be started soon after the diagnosis of POI and should be continued until the age of natural menopause.

Loss of ovarian activity can reduce total androgen production by 50%, which can affect sexual health. Therefore, if needed, androgens can be replaced in the form of testosterone cream/gel/implant/patch.

Combined Oral Contraception versus Hormone Replacement Therapy

There are no large, well controlled trials to compare combined oral contraception (COC) and HRT for the treatment of POI. As COC is

much more potent than HRT, such a strong action is not required for the treatment of POI. However, it is easy to take and is associated with less stigma. There are only a few trials comparing the effect of HRT and COC, but these studies are grossly underpowered to draw any meaningful conclusion. Langrish et al.[44] compared HRT (transdermal oestradiol 100–150 µg/day and vaginal progesterone 400 mg/day) versus COC (30 µg ethinyl oestradiol with 1.5 mg norethisterone) in a randomised, controlled, crossover trial and found that treatment with HRT resulted in lower mean 24 hour systolic and diastolic blood pressure over 1 year when compared to COC. Women who were on HRT had increased BMD and bone formation markers when compared to women who were given COC.[45] Cartwright et al.[46] also found that HRT administration (2 mg of 17-β-oestradiol and 75 µg levonorgestrel) significantly increased lumbar spine BMD at 2 years compared to COC administration (30 µg ethinyl oestradiol with 150 µg levonorgestrel) in patients with POI. Treatment with oestrogen and progesterone can be given either as either HRT or as COC until the results of larger randomised trials are available.

Side Effects of Hormone Replacement Therapy

In menopausal women, oral oestrogens increase the risk of VTE, the risk being the highest in the first year of its use. However, whether this data from older women can be extrapolated to young women still remains unanswered. Nevertheless, in order to reduce the risk of VTE, a transdermal route is preferred over an oral route.[43,47]

There is insufficient data to evaluate the association between oestrogen therapy given to women with POI and risk of developing breast cancer. A large population-based study including data from Danish Cancer Registry shows that the risk of breast cancer was increased in women on HRT aged ≥50 years, whereas the risk was not increased in women <50 years of age.[48] To prevent the risk of endometrial hyperplasia and carcinoma, progesterone should always be included in HRT to avoid unopposed action of oestrogens.

Other Measures for Bone Health

Bisphosphonates work by inhibiting the activity of osteoclasts and thus reduce bone resorption. However, it is not preferred in women of reproductive age group who are desirous of future pregnancy. Moreover, it is associated with osteonecrosis of the jaw and subtrochanteric fracture of the femur if taken for a long time. Weight bearing exercises should be done along with avoidance of smoking tobacco and drinking alcohol. Vitamin D status should also be measured and replenished if it is <30 IU/L. Calcium should also be supplemented in the dose of 800–1,000 mg/ day.[18]

Fertility Regulation

There are various treatment options to improve fertility in these women. In 5–10% of these women, there is spontaneous resumption of ovulation which can lead to pregnancy.[49] Thus, women not willing for future pregnancy should be offered a definite contraception.

Management of Infertility

The 'wait and see' approach is not appropriate for infertility treatment in patients with POI because ovulation is intermittent and unpredictable in these patients. However, spontaneous pregnancy with idiopathic POI is not associated with increased risk of miscarriage and obstetric complications.[18] A mixed retrospective and prospective study showed that 24% of women with idiopathic POI resumed ovarian function and the majority of the patients had resumption within 1 year of diagnosis.[50]

Pre-treatment with oestrogen suppresses FSH levels and allows restoration of FSH receptors in remaining follicles. Pre-treatment with oestrogen followed by exogenous gonadotropin stimulation resulted in ovulation in 32% of cases and pregnancy in 16% cases.[51] *In vitro* fertilisation with donor oocyte has the highest chance of pregnancy. In this, endometrium can be prepared for implantation by escalating the dose of oestradiol valerate along with natural progesterone. Pregnancy with donor oocyte is associated with complications such as hypertensive disorder, growth restriction, and preterm delivery.[52] It has been postulated that immunologic intolerance between mother and fetus is responsible for the complications.

Dehydroepiandrosterone promotes activation of oocytes and inhibits their atresia. Higher pregnancy rates with dehydroepiandrosterone supplementation has been noted in patients who have diminished ovarian function.[53]

Other new options are ovarian cortex transplantation and transplantation of an entire ovary.[54,55] Auto-transplant in cancer patients may be associated with the risk of dissemination. Thus, some authors have tried transplantation in monozygotic twins discordant for ovarian failure.[54] *In vitro* maturation of oocytes derived from preantral follicles which are spared in POI can be used as one of the treatment options.[1]

Ovarian Preservation[56,57]

To avoid follicular damage in young women requiring chemotherapy or radiotherapy for cancer, the following measures can be taken:

1. Gonadal shielding

2. Ovarian transposition

In this technique, ovaries are moved out of radiation field by cutting the utero-ovarian ligaments and mobilising them in patients undergoing radiotherapy for cancer management. The procedure has certain complications which include chronic pelvic pain and post-operative adhesions. Sometimes, the procedure itself may cause premature ovarian failure. Ovarian transpositioning prevents the ovaries from radiation induced injury only. Therefore, if a patient requires both chemotherapy and radiotherapy, this should be avoided.

3. Ovarian suppression by GnRH analogues

GnRH agonism suppresses FSH and luteinising hormone levels and protects the ovarian follicles from destruction by the chemotherapy by suspending ovarian function temporarily. Therefore, women in the reproductive age group should receive a GnRH agonist along with chemotherapy.

4. Cryopreservation of oocyte/embryo/ovarian tissue

(i) Embryo/oocyte cryopreservation:

Both embryo/oocyte cryopreservation requires controlled ovarian hyperstimulation and subsequent oocyte retrieval. This procedure demands delay of a minimum of 14 days before starting chemotherapy. To preserve embryos, *in vitro* fertilisation with either male partner or donor sperm is needed. Embryo cryopreservation has a good success rate in terms of cumulative pregnancy rates. Oocyte cryopreservation is more useful when a patient does not have partner. Oocytes are retrieved after ovarian stimulation and stored for future purposes. The cryopreservation of embryos and oocytes has a disadvantage that only a limited number can be stored at one time, thus limiting the number of attempts for future pregnancies.

(ii) Ovarian tissue cryopreservation:

This method requires ovarian tissue harvesting by laparoscopic procedure and storage of the tissue for future purposes. The advantage of this technique is that it does not demand hormonal stimulation of the ovary and there is no significant delay in initiation of chemotherapy.

Contraception

Because 5–10% of women with POI can have spontaneous resumption of ovulation, contraception should be provided to women not desirous of future fertility. Although combined oral contraceptive pills are one of the most commonly followed methods of contraception, they may fail to suppress the high FSH levels seen in POI and thus can result in pregnancy. There have been anecdotal reports of women with POI having conceived after taking oral contraceptive pills.[58] Barrier contraception or intrauterine devices are better options for these women.[20]

Social and Psychological Support

Many women with POI are not satisfied with the amount of information shared with them by their clinicians.[59] Family should be involved at the time of breaking bad news. Psychological support should be given in the form of counselling to the patients and appropriate treatment to those suffering from anxiety or depression. The diagnosis of POI, and its effects, can alter their quality of life. Large support groups for such women are available, such as the Daisy Network Premature Menopause Support Group and the International Premature Ovarian Failure Association. Considering the sensitive nature

of diagnoses, as well as cultural significance, patients should be informed of the diagnosis in a gentle manner, ensuring family support is available. Parents of adolescents with POI should also be counselled regarding the consequences and must be primed for providing emotional support to their daughters.[23]

FUTURE RESEARCH

An international research consortium and disease registry should be formed to provide clinical data regarding pathogenesis and management of POI.[60] Transplantation of ovarian cortex and activation of dormant follicles using a *PTEN* inhibitor in the re-implanted tissue needs further research. Large, randomised, controlled trials are required to compare various HRT options for their safety and efficacy. Until then, a multidisciplinary approach is the need of the era to identify such patients and provide them with optimum care with the utmost psychological support.

References

1. Goswami D, Conway GS. Premature ovarian failure. Horm Res. 2007;68(4):196-202.
2. Coulam CB et al. Incidence of premature ovarian failure. Obstet Gynecol. 1986;67(4):604-6.
3. Anasti JN. Premature ovarian failure: An update. Fertil Steril. 1998;70(1):1-15.
4. Himelstein-Braw R et al. Follicular atresia in the infant human ovary. J Reprod Fertil. 1976;46(1):55-9.
5. Persani L et al. Genes involved in human premature ovarian failure. J Mol Endocrinol. 2010;45(5):257-79.
6. Portnoï MF et al. Molecular cytogenetic studies of Xq critical regions in premature ovarian failure patients. Hum Reprod. 2006;21(9):2329-34.
7. Sala C et al. Eleven X chromosome breakpoints associated with premature ovarian failure (POF) map to a 15-Mb YAC contig spanning Xq21. Genomics. 1997;40(1):123-31.
8. Di Pasquale E et al. Hypergonadotropic ovarian failure associated with inherited mutation of human bone morphogenetic protein–15 (BMP15) gene. Am J Hum Genet. 2004;75(1):106-11.
9. Wiecker P. Genetic aspects of Premature ovarian failure. J Reprod Med Endocimol. 2009;6(1):17-8.
10. Hoek A et al. Premature ovarian failure and ovarian autoimmunity. Endocr Rev. 1997;18(1):107-34.
11. Falorni A et al. Steroid-cell autoantibodies are preferentially expressed in women with premature ovarian failure who have adrenal autoimmunity. Fertil Steril. 2002;78(2):270-9.
12. Novosad JA et al. Ovarian antibodies as detected by indirect immunofluoroscence are unreliable in diagnosis of autoimmune premature ovarian failure: A controlled evaluation. BMC Women Health. 2003;3(1):2.
13. Morrison JC et al. Mumps oophoritis: A cause of premature menopause. Fertil Steril. 1975;26(7):655-9.
14. Lushbaugh CC, Casarett GW. The effects of gonadal irradiation in clinical radiation therapy: A review. Cancer. 1976;37(Suppl2):1111-25.
15. Larsen EC et al. Reduced ovarian function in long-term survivors of radiation- and chemotherapy- treated childhood cancer. J Clin Endocrinol Metab. 2003;88(11):5307-14.
16. Vegetti W et al. Inheritance in idiopathic premature ovarian failure: Analysis of 71 cases. Hum Reprod. 1998;13(7):1796-800.
17. Schover LR. Premature ovarian failure and its consequences: Vasomotor symptoms, sexuality, and fertility. J Clin Oncol. 2008;26(5):753-8.
18. Hamoda H. The British Menopause Society and Women's Health Concern Recommendations on the management of women with premature ovarian insufficiency. Post Reprod Health. 2017;23(1):22-35.
19. Bachelot A et al. Phenotyping and genetic studies of 357 consecutive patients presenting with premature ovarian failure. Eur J Endocrinol. 2009;161(1):179-87.
20. Nelson LM. Clinical Practice. Premature ovarian insufficiency. N Engl J Med. 2009;360(6):606-14.
21. Rebar RW et al. Idiopathic premature ovarian failure: Clinical and endocrine characteristics. Fertil Steril. 1982;37(1):35-41.
22. Webber L et al.; European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI. ESHRE guidelines: Management of women with premature ovarian insufficiency. Hum Reprod. 2016;31(5):926-37.
23. American College of Obstetricians and Gynecologists. Committee opinion no 605: Primary ovarian failure in adolescents and young women. Obstet Gynaecol. 2014;124(1):193-7.
24. Rebar RW, Connolly HV. Clinical features of young women with hypergonadotropic amenorrhea. Fertil Steril. 1990;53(5):804-10.
25. Lebovic DI, Naz R. Premature ovarian failure: Think "autoimmune disorder". Sex Reprod Menopause. 2004;2(4):230-3.
26. Kim TJ et al. Routine endocrine screening for patients with karyotypically normal spontaneous premature ovarian failure. Obstet Gynaecol. 1997;89(5Pt1):777-9.
27. Baber RJ et al.; The IMS Writing Group. 2016 IMS Recommendations on women's midlife health and menopause hormone therapy. Available at: https://www.imsociety.org/manage/images/pdf/4429e3dd302aac259ad68c3be7f60599.pdf. Last accessed: 1 May 2019.
28. Atsma F et al. Postmenopausal status and early menopause as independent factors for cardiovascular disease: A meta-analysis. Menopause. 2006;13(2):265-79.
29. Rocca WA et al. Survival patterns after oophorectomy

in premenopausal women: A population-based cohort study. Lancet Oncol. 2006;7(10):821-8.

30. Gordon T et al. Menopause and coronary heart disease. The Framingham Study. Ann Intern Med. 1978;89(2):157-61.
31. Anasti JN et al. Bone loss in young women with karyotypically normal spontaneous premature ovarian failure. Obstet Gynecol. 1998;91(1):12-5.
32. Vega EM et al. Influence of the menopausal age on the severity of osteoporosis in women with vertebral fractures. Maturitas. 1994;19(2):117-24.
33. Rocca WA et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. Neurology. 2007;69(11):1074-83.
34. Bove R et al. Age at surgical menopause influences cognitive decline and Alzheimer pathology in older women. Neurology. 2014;82(3):222-9.
35. Graziottin A, Basson R. Sexual dysfunction in women with premature menopause. Menopause. 2004;11(6Pt2):766-77.
36. Yela DA et al. Influence of sexual function on the social relations and quality of life of women with premature ovarian insufficiency. Rev Bras Ginecol Obstet. 2018;40(2):66-71.
37. Schmidt PJ et al. Depression in women with spontaneous 46, XX primary ovarian insufficiency. J Clin Endocrinol Metab. 2011;96(2):E278-87.
38. Davis M et al. The psychosocial transition associated with spontaneous 46, XX primary ovarian insufficiency: Illness uncertainty, stigma, goal flexibility, and purpose in life as factors in emotional health. Fertil Steril. 2010;93(7):2321-9.
39. Orshan SA et al. Women with spontaneous 46, XX primary ovarian insufficiency (hypergonadotropic hypogonadism) have lower perceived social support than control women. Fertil Steril. 2009;92(2):688-93.
40. Benetti-Pinto CL et al. Quality of life in women with premature ovarian failure. Gynecol Endocrinol. 2011;27(9):645-9.
41. Rossouw JE et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA. 2007;297(13):1465-77.
42. van der Klift M et al. Risk factors for incident vertebral fractures in men and women: The Rotterdam Study. J Bone Miner Res. 2004;19(7):1172-80.
43. American College of Obstetricians and Gynecologists. ACOG committee opinion no 556: Postmenopausal estrogen therapy: Route of administration and risk of venous thromboembolism. Obstet Gynecol. 2013;121(4):887-90.
44. Langrish JP et al. Cardiovascular effects of physiological and standard sex steroid replacement regimens in premature ovarian failure. Hypertension. 2009;53(5):805-11.
45. Crofton PM et al. Physiological versus standard sex steroid replacement in young women with premature ovarian failure: Effects on bone mass acquisition and turnover. Clin Endocrinol (Oxf). 2010;73(6):707-14.
46. Cartwright B et al. Hormone replacement therapy versus the combined oral contraceptive pill in premature ovarian failure: A randomized controlled trial of the effects on bone mineral density. J Clin Endocrinol Metab. 2016;101(9):3497-505.
47. National Institute for Health and Care Excellence. Menopause: Diagnosis and management. NICE guideline [NG23]. Available at: https://www.nice.org.uk/guidance/ng23/chapter/Recommendations#diagnosing-and-managing-premature-ovarian-insufficiency. Last accessed: 1 May 2019.
48. Ewertz M et al. Hormone use for menopausal symptoms and risk of breast cancer: A Danish cohort Study. Br J Cancer. 2005;92(7):1293-7.
49. van Kasteren YM, Schoemaker J. Premature ovarian failure: A systematic review on therapeutic interventions to restore ovarian function and achieve pregnancy. Hum Reprod Update. 1999;5(5):483-92.
50. Bidet M et al. Resumption of ovarian function and pregnancies in 358 patients with premature ovarian failure. J Clin Endocrinol Metab. 2011;96(12):3864-72.
51. Tartagni M et al. Effects of pretreatment with estrogens on ovarian stimulation with gonadotropins in women with premature ovarian failure: A randomized placebo-controlled trial. Fertil Steril. 2007;87(4):858-61.
52. Jeve YB et al. Donor oocyte conception and pregnancy complications: A systemic review and meta-analysis. BJOG. 2016;123(9):1471-80.
53. Barad D, Gleicher N. Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. Hum Reprod. 2006;21(11):2845-9.
54. Silber SJ, Gosden RG. Ovarian transplantation in a series of monozygotic twins discordant for ovarian failure. N Engl J Med. 2007;356(13):1382-4.
55. Silber SJ et al. Successful pregnancy after microsurgical transplantation of an intact ovary. N Engl J Med. 2008;359(24):2617-8.
56. Mahajan N. Fertility preservation in female cancer patients: An overview. J Hum Reprod Sci. 2015;8(1):3-13.
57. Taylan E, Oktay KH. Current state and controversies in fertility preservation in women with breast cancer. World J Clin Oncol. 2017;8(3):241-8.
58. Alper MM et al. Pregnancies after premature ovarian failure. Obstet Gynecol. 1986;67(3Suppl):59-62S.
59. Groff AA et al. Assessing the emotional needs of women with spontaneous premature ovarian failure. Fertil Steril. 2005;83(6):1734-41.
60. Cooper AR et al. The time is now for a new approach to premature ovarian insufficiency. Fertil Steril. 2011;95(6):1890-7.

Deep Learning Strategies for Ultrasound in Pregnancy

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Abstract

Ultrasound is one of the most ubiquitous imaging modalities in clinical practice. It is cheap, does not require ionising radiation, and can be performed at the bedside, making it the most commonly used imaging technique in pregnancy. Despite these advantages, it does have some drawbacks such as relatively low imaging quality, low contrast, and high variability. With these constraints, automating the interpretation of ultrasound images is challenging. However, successfully automated identification of structures within three-dimensional ultrasound volumes has the potential to revolutionise clinical practice. For example, a small placental volume in the first trimester is correlated to adverse outcome later in pregnancy. If the placenta could be segmented reliably and automatically from a static three-dimensional ultrasound volume, it would facilitate the use of its estimated volume, and other morphological metrics, as part of a screening test for increased risk of pregnancy complications, potentially improving clinical outcomes.

Recently, deep learning has emerged, achieving state-of-the-art performance in various research fields, notably medical image analysis involving classification, segmentation, object detection, and tracking tasks. Due to its increased performance with large datasets, deep learning has garnered great interest relating to medical imaging applications. In this review, the authors present an overview of deep learning methods applied to ultrasound in pregnancy, introducing their architectures and analysing strategies. Some common problems are presented and some perspectives into potential future research are provided.

INTRODUCTION

In medical imaging, the most commonly employed deep learning methods are convolutional neural networks (CNN).[1-8] Compared to classical machine learning algorithms, CNN have enabled the development of numerous solutions not previously achievable because they do not need a human operator to identify an initial set of features: they can find relevant

features within the data itself. In many cases, CNN are better able to identify features than the human eye.

CNN have some disadvantages however: they need large amounts of data to automatically find the right features and processing large datasets is both computationally costly and takes time. Fortunately, training time can be reduced significantly if parallel architectures are used (e.g., by using graphics cards).

In medical imaging, deep learning is increasingly used for tasks such as automated lesion detection, segmentation, and registration to assist clinicians in disease diagnosis and surgical planning. Deep learning techniques have the potential to create new screening tools, predict diseases, improve diagnostic accuracy, and accelerate clinical tasks, whilst also reducing costs and human error.[9–17] For example, automated lesion segmentation tools usually run in a few seconds, much faster than human operators, and often provide more reproducible results.

Ultrasound is the most commonly used medical imaging modality for diagnosis and screening in clinical practice.[18] It presents many advantages over other modalities such as X-ray, magnetic resonance imaging (MRI), and computed tomography (CT) because it does not use ionising radiation, is portable, and is relatively cheap.[19] However, ultrasound has its disadvantages. It often has relatively low imaging quality, is prone to artefacts, is highly dependent on operator experience, and has high inter- and intra-observer variability across different manufacturers' machines.[10] Nonetheless, its safety profile, noninvasive nature, and convenience makes it the primary imaging modality for fetal assessment in pregnancy.[20] This includes early pregnancy dating, screening for fetal structural abnormalities, and the estimation of fetal weight and growth velocity.[21] Although two-dimensional (2D) ultrasound is most commonly used for pregnancy evaluation due to its wide availability and high resolution, most machines also have three-dimensional (3D) probes and software, which have been successfully employed to detect fetal structural abnormalities.[22]

Ultrasound has a number of limitations when it comes to intrauterine scanning, including small field-of-view, poor image quality under certain conditions (e.g., reduced amniotic fluid), limited soft-tissue acoustic contrast, and beam attenuation caused by adipose tissue.[22] Furthermore, fetal position, gestational age-induced effects (poor visualisation, skull ossification), and fetal tissue definition can also affect the assessment.[20] As a result, a high level of expertise is essential to ensure adequate image acquisition and appropriate clinical diagnostic performance. Thus, ultrasound examination results are highly dependent on the training, experience, and skill of the sonographer.[23]

A study of the prenatal detection of malformations using ultrasound images demonstrated that the performance sensitivity ranged from 27.5% to 96.0% among different medical institutions.[24] Even when undertaken correctly by an expert, manual interrogation of ultrasound images is still time-consuming and this limits its use as a population-based screening tool.

To address these challenges, automated image analysis tools have been developed which are able to provide faster, more accurate, and less subjective ultrasound markers for a variety of diagnoses. In this paper, the authors review some of the most recent developments in deep learning which have been applied to ultrasound in pregnancy.

DEEP LEARNING APPLICATIONS IN PREGNANCY ULTRASOUND

Deep learning techniques have been used for ultrasound image analysis in pregnancy to address such tasks as classification, object detection, and tissue segmentation. This review covers applications in pregnancy. The reviewed papers were identified with a broad free-text search on the most commonly used medical databases (PubMed, Google Scholar etc.). The search was augmented by reviewing the references in the identified papers. The resulting papers were assessed by the authors and filtered for perceived novelty, impact in the field, and published date (2017-2020). Table 1 lists the literature reviewed in this section.

Fetal Segmentation

Ultrasound is the imaging modality most commonly used in routine obstetric examination.

Table 1: Literature reviewed.

Publication	Objective	Approach
Fetal segmentation		
Namburete et al.[9] (2018)	Segmentation and alignment (brain)	Modified FCN
Torrents-Barrena et al.[25] (2019)	Segmentation (whole fetus)	Several approaches
Philip et al.[26] (2019)	Segmentation and measurement (heart)	3D-U-net
Al-Bander et al.[11] (2020)	Segmentation (head)	Mask-RCNN + Resnet
Placental segmentation		
Qi et al.[27] (2017)	Anatomy recognition	ResNet
Looney et al.[28] (2017)	Segmentation	Parallel CNN
Looney et al.[29] (2018)	Segmentation	OxNNet
Oguz et al.[30] (2018)	Segmentation	CNN
Yin et al.[31] (2020)	Anatomy recognition	Multi-class FCN
Hu et al.[32] (2019)	Segmentation	Modified U-Net
Torrents-Barrena et al.[33] (2019)	Segmentation	CGAN
Zimmer et al.[34] (2019)	Segmentation	3D CNN

CGAN: conditional generative adversarial network; CNN: convolutional neural networks; FCN: fully convolutional neural network; RCNN: Regional Convolutional Neural Network.

Fetal segmentation and volumetric measurement have been explored for many applications, including assessment of the fetal health, calculation of gestational age, and growth velocity. Ultrasound is also used for structural and functional assessment of the fetal heart, head biometrics, brain development, and cerebral abnormalities. This antenatal assessment allows clinicians to make an early diagnosis of many conditions, facilitating parental choice and enabling appropriate planning for the rest of the pregnancy including early delivery.

Currently, fetal segmentation and volumetric measurement still rely on manual or semi-automated methods, which are time-consuming and subject to inter-observer variability.[11] Effective fully automated segmentation is required to address these issues. Recent developments to facilitate automated fetal segmentation from 3D ultrasound are presented below:

Namburete et al.[9] developed a methodology to address the challenge of aligning 3D ultrasound images of the fetal brain to form the basis of automated analysis of brain maturation. A multi-task fully convolutional neural network (FCNN) was used to localise the 3D fetal brain, segment structures, and then align them to a referential co-ordinate system. The network was optimised by simultaneously learning features shared within the input data pertaining to the correlated tasks, and later branching out into task-specific output streams.

The proposed model was trained on a dataset of 599 volumes with a gestational age ranging from 18 to 34 weeks, and then evaluated on a clinical dataset consisting of 140 volumes presenting both healthy and growth-restricted fetuses from different ethnic and geographical groups. The automatically co-aligned volumes showed a good correlation between fetal anatomies.

Torrents-Barrena et al.[25] proposed a radiomics-based method to segment different fetal tissues from MRI and 3D ultrasound. This is the first time that radiomics (the high-throughput extraction of large numbers of image features from radiographic images[35]) has been used for segmentation purposes. First, handcrafted radiomic features were extracted to characterise the uterus, placenta, umbilical cord, fetal lungs, and brain. Then the radiomics for each anatomical target were optimised using both K-best and Sequential Forward Feature Selection techniques. Finally, a Support Vector Machine with instance balancing was adopted for accurate segmentation using these features as its input. In addition, several state-of-the-art deep learning-based segmentation approaches were studied and validated on a set of 60 axial MRI and 3D ultrasound images from pathological and clinical cases. Their results demonstrated that a combination of 10 selected radiomic features led to the highest tissue segmentation performance.

Philip et al.[26] proposed a 3D U-Net based fully automated method to segment the fetal annulus (base of the heart valves). The aim of this was to build a tool to help fetal medicine experts with assessment of fetal cardiac function. The method was trained and tested on 250 cases (at different points in the cardiac cycle to ensure that the technique was valid). This provided automated measurements of the excursion of the mitral and tricuspid valve annular planes in form of TAPSE/MAPSE (TAPSE: tricuspid annular plane systolic excursion; MAPSE: mitral annular plane systolic excursion). This demonstrated the feasibility of using this technique for automated segmentation of the fetal annulus.

Al-Bander et al.[11] introduced a deep learning-based method to segment the fetal head in ultrasound images. The fetal head boundary was detected by incorporating an object localisation scheme into the segmentation, achieved by combining a Mask R-CNN (Regional Convolutional Neural Network) with a FCNN. The proposed model was trained on 999 3D ultrasound images and tested on 335 images captured from 551 pregnant women with a gestational age ranging between 12 and 20 weeks. Finally, an ellipse was fitted to the contour of the detected fetal head using the least-squares fitting algorithm.[36] Figure 1 illustrates the examples of fetal head segmentation.

Placental Segmentation

The placenta is an essential organ which plays a vital role in the healthy growth and development of the fetus. It permits the exchange of respiratory gases, nutrients, and waste between mother and fetus. It also synthesises many substances that maintain the pregnancy, including oestrogen, progesterone, cytokines, and growth factors. Furthermore, the placenta also functions as a barrier, protecting the fetus against pathogens and drugs.[37]

Abnormal placental function affects the development of the fetus and causes obstetric complications such as pre-eclampsia. Placental insufficiency is associated with adverse pregnancy outcomes including fetal growth restriction, caused by insufficient transport of nutrients and oxygen through the placenta.[38] A good indicator of future placental function is the size of the placenta in early pregnancy. The placental volume as early as 11 to 13 weeks' gestation has long been known to correlate with birth weight at term.[39] Poor vascularity of the first-trimester placenta also increases the risk of developing pre-eclampsia later in pregnancy.[40]

Reliable placental segmentation is the basis of further measurement and analysis which has the ability to predict adverse outcomes. However, full automation of this is a challenging task due to the heterogeneity of ultrasound images, indistinct boundaries, and the placenta's variable shape and position. Manual segmentation is relatively accurate but is extremely time-consuming. Semi-automated image analysis tools are faster but are still time-consuming and typically require the operator to manually identify the placenta within the image. An accurate and fully automated technique for placental segmentation that provides measurements such as placental volume and vascularity would permit population-based screening for pregnancies at risk of adverse outcomes. Figure 2 illustrates an example of placenta segmentation.

Qi et al.[27] proposed a weakly supervised CNN for anatomy recognition in 2D placental ultrasound images. This was the first successful attempt at multi-structure detection in placental ultrasound images.

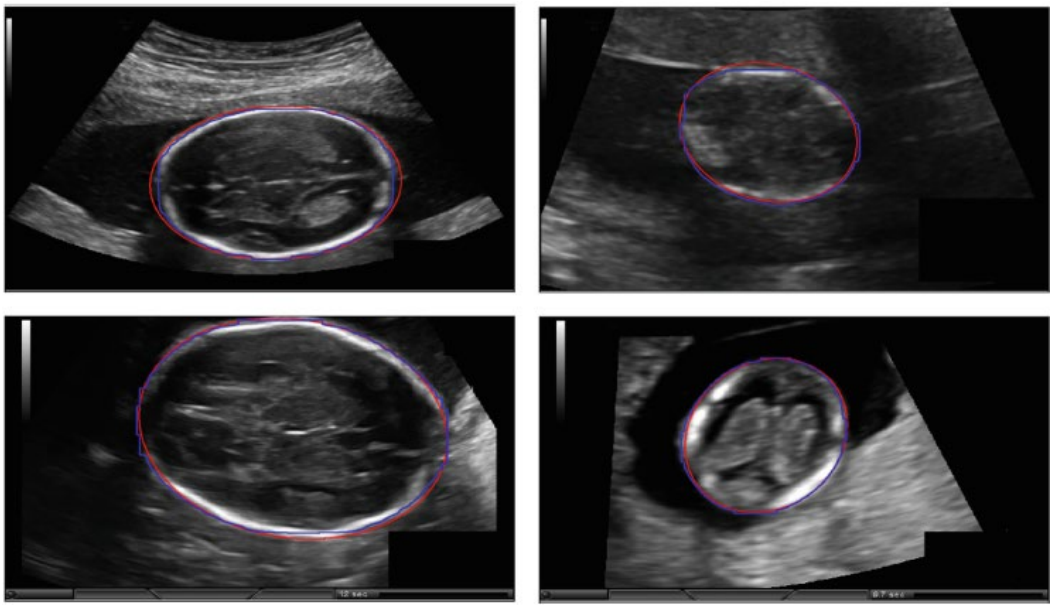

Figure 1: Examples of fetal head segmentation showing the ellipse fitted results on the two-dimensional ultrasound sections.

Manual annotation (blue); automated segmentation (red).

Adapted from Al-Bander et al.[11]

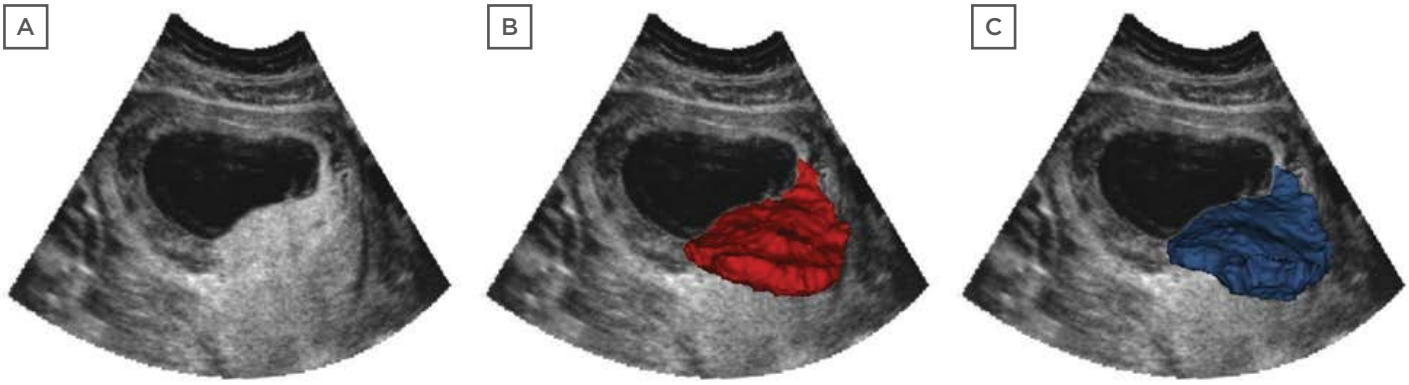

Figure 2: Placenta segmentation of first-trimester pregnancy: A) two-dimensional B-mode plane; B) semi-automated Random Walker result; C) OxNNet prediction result.

Adapted from Looney et al.[29]

The CNN was designed to learn discriminative features in Class Activation Maps (one for each class), which are generated by applying Global Average Pooling in the last hidden layer. An image dataset of 10,808 image patches from 60 placental ultrasound volumes were used to evaluate the proposed method. Experimental results demonstrated that the proposed method achieved high recognition accuracy, and could localise complex anatomical structures around the placenta.

Looney et al.[28] used a CNN named *DeepMedic*[41] to automate segmentation of placenta in 3D ultrasound. This was the first attempt to segment 3D placental ultrasound using a CNN. Their database contained 300 3D ultrasound volumes from the first trimester. The placenta was segmented in a semi-automated manner using the Random Walker method,[42] to provide a 'ground truth' dataset. The results of the *DeepMedic* CNN were compared against semi-automated segmentation, achieving median Dice

similarity coefficient (DSC) of 0.73 (first quartile, third quartile: 0.66, 0.76) and median Hausdorff distance of 27 mm (first quartile, third quartile: 18 mm, 36 mm).

Looney et al.[29] then presented a new 3D FCNN named *OxNNet*. This was based on the 2D U-net architecture to fully automate segmentation of the placenta in 3D ultrasound volumes. A large dataset, composed of 2,393 first trimester 3D ultrasound volumes, was used for training and testing purposes. The ground truth dataset was generated using the semi-automated Random Walker method[42] (initially seeded by three expert operators). The *OxNNet* FCNN obtained placental segmentation with state-of-the-art accuracy (median DSC of 0.84, interquartile range 0.09). They also demonstrated that increasing the size of the training set improves the performance of the FCNN. In addition, the placental volumes segmented by *OxNNet* were correlated with birth weight to predict small-for-gestational-age babies, showing almost identical clinical conclusions to those produced by the validated semi-automated tools.

Oguz et al.[30] combined a CNN with multi-atlas joint label fusion and Random Forest algorithms for fully automated placental segmentation. A dataset of 47 ultrasound volumes from the first trimester was pre-processed by data augmentation. The resulting dataset was used to train a 2D CNN to generate a first 3D prediction. This was used to build a multi-atlas joint label fusion algorithm, generating a second prediction. These two predictions were fused together using a Random Forest algorithm, enhancing overall performance. A four-fold cross-validation was performed and the proposed method reportedly achieved a mean Dice coefficient of 0.863 (±0.053) for the test folds.

Yin et al.[31] proposed a fully automated method combining deep learning and image processing techniques to extract the vasculature of the placental bed from 3D power Doppler ultrasound scans and estimate its perfusion. A multi-class FCNN was applied to separate the placenta, amniotic fluid, and fetus from the 3D ultrasound volume to provide accurate localisation of the utero-placental interface (UPI) where the maternal blood enters the placenta from the uterus. A transfer learning technique was applied to initialise the model using parameters optimised by a single-class model[29] trained on 1,200 labelled placental volumes. The vasculature was segmented by a region growing algorithm from the 3D power Doppler signal. Based on the representative vessels at a certain distance from the UPI, the perfusion of placental bed was estimated using a validated technique known as FMBV (fractional moving blood volume).[43]

Hu et al.[32] proposed a FCNN based on the U-net architecture for 2D placental ultrasound segmentation. The U-net had a novel convolutional layer weighted by automated acoustic shadow detection, which helped to recognise ultrasound artefacts. The dataset used for evaluation contained 1,364 fetal ultrasound images acquired from 247 patients over 47 months. The dataset was diverse because the image data was acquired from different machines operated by different specialists and presented scanning of fetuses at different gestational ages. The proposed method was first applied across the entire dataset and then over a subset of images containing acoustic shadows. In both cases, the acoustic shadow detection scheme was proven to be able to improve segmentation accuracy.

Torrents-Barrena et al.[33] proposed the first fully automated framework to segment both the placenta and the fetoplacental vasculature in 3D ultrasound, demonstrating that ultrasound enables the assessment of twin-to-twin transfusion syndrome by providing placental vessel mapping. A conditional Generative Adversarial Network was adopted to identify the placenta, and a Modified Spatial Kernelized Fuzzy C-Means combined with Markov Random Fields was used to extract the vasculature. The method was applied on a dataset of 61 ultrasound volumes, which was heterogeneous due to different placenta positions, in singleton or twin pregnancies of 15 to 38 weeks' gestation. The results achieved a mean Dice coefficient of 0.75±0.12 for the placenta and 0.70±0.14 for its vessels on images that had been pre-processed by down-sampling and cropping.

Zimmer et al.[34] focussed on the placenta at late gestational age. Ultrasound scans are typically useful only in the early stages of pregnancy because a limited field of view only permits the complete capture of small placentas. To overcome this, a multi-probe system was used to acquire different fields of view and then combine them with a voxel-wise fusion algorithm to obtain

a fused ultrasound volume capturing the whole placenta. The dataset used for evaluation was composed of 127 single 4D (3D+time) ultrasound volumes from 30 patients covering different parts of the placenta. In total, 42 fused volumes were derived from these simple volumes which extended the field of view. Both the simple and fused volumes were used for evaluation of their 3D CNN based automated segmentation. The best results of placental volume segmentation were comparable to corresponding volumes extracted from MRI, achieving Dice coefficient of 0.81±0.05.

DISCUSSION

The number of applications for deep learning in pregnancy ultrasound has increased rapidly over the last few years and they are beginning to show very promising results. Along with new advances in deep learning methods, new ultrasound applications are being developed to improve computer-aided diagnosis and enable the development of automated screening tools for pregnancy.

A number of deep learning algorithms have been presented in this review, showing novel approaches, state-of-the-art results, and pioneering applications that have contributed so far to the pregnancy ultrasound analysis. Some methods rely on sophisticated hybrid approaches, combining different machine learning or image analysis techniques, whilst others rely on smart manipulation of the dataset such as fusing volumes or applying data augmentation. Large quality-controlled datasets are enabling single deep learning algorithms to be successfully developed still. However, it's not currently possible to compare these methods directly, even if designed for the same task, because they all use different datasets and measurements.

The technological advances in medical equipment and image acquisition protocols allow better data acquisition to enhance the trained models. The size and availability of quality-controlled ground-truth datasets remain significant issues to be addressed. The performance of deep learning methods usually depends on the number of samples. Most of the presented methods cannot be independently evaluated because their datasets are small and not widely available. In addition, models trained on one dataset might fail on another generated by a different manufacturer's machine. Large, publicly available, and appropriately quality-controlled ultrasound datasets are needed to compare different deep learning methods and achieve robust performance in real world scenarios.

There is also an urgent need to implement deep learning methods to solve relevant clinical problems. Very few papers translate the simple application of algorithms to a broader, practical solution that could be widely used in clinical practice. The practical implementation of deep learning methods and assessment of the correlation between automated results and clinical outcomes should be a focus of future research.

CONCLUSION

The field of deep learning in pregnancy ultrasound is still developing. Lack of sufficient high-quality data and practical clinical solutions are some of the key barriers. In addition, the newest deep learning methods tend to be applied first to other more homogeneous medical imaging modalities such as CT or MRI. Therefore, there is a need for researchers to collaborate across modalities to transfer existing deep learning algorithms to the field of pregnancy ultrasound to achieve better performance and create new applications in the future.

References

1. Simonyan K, Zisserman A. Very deep convolutional networks for largescale image recognition. arXiv preprint arXiv. 2014;1409.1556.
2. Szegedy C et al. Going deeper with convolutions. In: proceedings of the IEEE conference on computer vision and pattern recognition. 2014;arXiv:1409.4842:1-9.
3. He K et al. Deep residual learning for image recognition. In: proceedings of the IEEE conference on computer vision and pattern recognition. 2015;arXiv:1512.03385:770-8.
4. Huang G et al. Densely

connected convolutional networks. In: proceedings of the IEEE conference on computer vision and pattern recognition. 2016;arXiv:1608.06993:4700-8.

5. Hu J et al. Squeeze-and-excitation networks. In: proceedings of the IEEE conference on computer vision and pattern recognition. 2018;arXiv:1709.01507:7132-41.
6. Wang D et al. Exploring linear relationship in feature map subspace for convnets compression. 2018;arXiv:1803.05729.
7. Ren S et al. Faster r-cnn: towards real-time object detection with region proposal networks. In: advances in neural information processing systems. 2015;arXiv:1506.01497:91-9.
8. He K et al. Spatial pyramid pooling in deep convolutional networks for visual recognition. In: IEEE transactions on pattern analysis and machine intelligence. 2015;37(9):1904-16.
9. Namburete A et al. Fully automated alignment of 3d fetal brain ultrasound to a canonical reference space using multi-task learning. Med Image Anal. 2018;46:1-14.
10. Liu S et al. Deep learning in medical ultrasound analysis: a review. Engineering. 2019;5(2):261-75.
11. Al-Bander B et al., "Improving fetal head contour detection by object localisation with deep learning", Zheng Y et al. (eds). Annual Conference on Medical Image Understanding and Analysis (2020), Springer, pp. 142-50.
12. Sa R et al. Intervertebral disc detection in x-ray images using faster R-CNN. Conf Proc IEEE Eng Med Biol Soc. 2017;2017:564-7.
13. Q Li et al., "Medical image classification with convolutional neural network," 2014 13th International Conference on Control Automation Robotics & Vision (ICARCV), Singapore, 2014, pp. 844-848, doi: 10.1109/ICARCV.2014.7064414.
14. Miao S et al., "Real-time 2D/3D registration via CNN regression," IEEE 13th International Symposium on Biomedical Imaging (2016). IEEE. 2016;arXiv:1507.07505:1430-4.
15. Diniz J et al. Spinal cord detection in planning CT for radiotherapy through adaptive template matching, IMSLIC and convolutional neural networks. Comput Methods Programs Biomed. 2019;170:53-67.
16. Diniz P et al. Detection of white matter lesion regions in MRI using SLIC0 and convolutional neural network. Comput Methods Programs Biomed. 2018;167:49-63.
17. Diniz J et al. Detection of mass regions in mammograms by bilateral analysis adapted to breast density using similarity indexes and convolutional neural networks. Comput Methods Programs Biomed. 2018;156:191-207.
18. Noble J, Boukerroui D. Ultrasound image segmentation: a survey. IEEE Trans Med Imaging. 2006;25(8): 987-1010.
19. Brattain L J et al. Machine learning for medical ultrasound: status, methods, and future opportunities. Abdom Radiol. 2018;43(4):786-99.
20. Rueda S et al. Evaluation and comparison of current fetal ultrasound image segmentation methods for biometric measurements: a grand challenge. IEEE Trans Med Imaging. 2013;33(4):797-813.
21. Reddy U et al. Prenatal imaging: ultrasonography and magnetic resonance imaging. Obstet and Gynecol. 2008;112(1):145-57
22. Roy-Lacroix M et al. A comparison of standard two-dimensional ultrasound to three-dimensional volume sonography for routine second-trimester fetal imaging. J Perinatol. 2017;37(4):380-6.
23. Sarris I et al. Intra- and interobserver variability in fetal ultrasound measurements. Ultrasound Obstet Gynecol. 2012;39(3):266-73.
24. Salomon L et al. A score-based method for quality control of fetal images at routine second-trimester ultrasound examination. Prenat Diagn. 2008;28(9):822-7.
25. Torrents-Barrena J et al. Assessment of radiomics and deep learning for the segmentation of fetal and maternal anatomy in magnetic resonance imaging and ultrasound. Acad Radiol. 2019;S1076-6332(19)30575-6.
26. Philip M et al. (2019). Convolutional Neural Networks for Automated Fetal Cardiac Assessment using 4D B-Mode Ultrasound. 824-828. 10.1109/ISBI.2019.8759377.
27. Qi H et al. Weakly supervised learning of placental ultrasound images with residual networks. Med Image Underst Anal Conf. 2017;723:98-108.
28. Looney P et al. Automatic 3D ultrasound segmentation of the first trimester placenta using deep learning. 2017 IEEE 14th International Symposium on Biomedical Imaging. 2017;279-282.
29. Looney P et al. Fully automated, real-time 3d ultrasound segmentation to estimate first trimester placental volume using deep learning. JCI Insight. 2018;3(11):e120178.
30. Oguz B et al. Combining deep learning and multi-atlas label fusion for automated placenta segmentation from 3DUS. Data Driven Treatment Response Assessment and Preterm, Perinatal, and Paediatric Image Analysis. Springer. 2018;138-48.
31. Yin Y et al. Standardization of blood flow measurements by automated vascular analysis from power doppler ultrasound scan. Medical Imaging 2020: Computer-Aided Diagnosis. International Society for Optics and Photonics. 2020;113144C:1025-30.
32. Hu R et al. Automated placenta segmentation with a convolutional neural network weighted by acoustic shadow detection. Conf Proc IEEE Eng Med Biol Soc. 2019;6718-23.
33. Torrents-Barrena J et al. Automatic segmentation of the placenta and its peripheral vasculature in volumetric ultrasound for TTTS fetal surgery. IEEE 16th International Symposium on Biomedical Imaging. 2019;772-5.
34. Zimmer V A et al. Towards whole placenta segmentation at late gestation using multi-view ultrasound images. Med Image Comput Comput Assist Interv. 2019;628-36.
35. Lambin P et al. Radiomics: extracting more information from medical images using advanced feature analysis. Eur J Cancer. 2012;48(4):441-6.
36. Stigler S. Gauss and the invention of least squares. The Annals of Statistics. 9 1981;9(3):465-74.
37. Han M et al. Automatic segmentation of human placenta images with U-net. IEEE Access. 2019;7:180083-92.
38. Salavati N et al. The possible role of placental morphometry in the detection of fetal growth restriction. Front Physiol. 2019;9:1884.
39. Salafia C et al. Placenta and fetal growth restriction. Clin Obstet Gynecol. 2006;49(2) 236-56.
40. Mathewlynn S, Collins S. Volume and vascularity: using ultrasound to unlock the secrets of the first trimester placenta. Placenta. 2019;84:32-6.
41. Kamnitsas K et al. Deepmedic for brain tumor segmentation. International workshop on Brain lesion: Glioma, Multiple sclerosis, Stroke and Traumatic Brain Injuries. 2016;138-149.
42. Grady L. Random walks for image segmentation. IEEE Transactions on Pattern Analysis and Machine Intelligence. 2006;28(11):1768-83.
43. Stevenson G et al. A technique for the estimation of fractional moving blood volume by using three-dimensional power doppler US. Radiology. 2015;274(1):230-7.

Successful Outcome of Cardiac Arrest Management in a Morbidly Obese Parturient Woman During Caesarean Section Delivery: A Case Report

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Abstract

During a caesarean section (CS), severe hypotension following spinal anaesthesia, aortocaval compression, and morbid obesity may cause a decrease in cardiac output resulting in cardiac arrest. Cardiopulmonary resuscitation during CS is stressful for all the attending team, which mandates the importance of a high level of skill and readiness to perform perimortem CS. Reported here is a case of 36-year-old, full-term, morbidly obese parturient female who developed cardiac arrest during an emergency CS under spinal anaesthesia before delivery of the baby. Cardiopulmonary resuscitation was performed and enhanced with resuming of left lateral tilt and completion of perimortem CS. A healthy baby was delivered, and the operation was completed with good homeostasis.

INTRODUCTION

Compression of the inferior vena cava and abdominal aorta by the gravid uterus in addition to hypotension following spinal anaesthesia during caesarean section (CS) may be enhanced by morbid obesity. It may result in a severe decrease in venous return (preload) and afterload; furthermore, a decrease in cardiac output may occur. Without early recognition and correction, cardiac arrest (CA) may ensue. The positioning of the uterus and abdomen during CS is critical to avoid aortocaval compression. It is achieved by tilting of the operation table to the left side or by placing a wedge under the right buttock of the patient. The left lateral tilt could make the operation more difficult; however, it facilitates effective cardiopulmonary resuscitation (CPR) and obstetricians should be familiar with delivering a baby in this positioning. CPR during CS is stressful for all attending staff, and mandates the importance of more skills and the ability to perform or complete a perimortem CS.[1-3]

CASE REPORT

A 36-year-old (gravida 3, para 2) full-term, morbidly obese parturient woman was planned for emergency CS as a result of a failure to progress after induction of labour. The patient

was lying in the left lateral position with a size 18-gauge intravenous cannula at the dorsum of the right hand and dextrose in normal saline was running. She had a body weight of 136 kg, height of 162 cm, and a BMI of 51.8. She was not diabetic, hypertensive, or asthmatic, and had irregular antenatal care. The bulk of her increasing body weight was during the prior 2 years. Her last meal was approximately 3 hours before and contained milk. Vital signs were a heart rate of 107 beats/min, blood pressure 125/78 mmHg, and O_2 saturation 95% (room air). No signs of fetal distress from the obstetric side were noted. A Foley catheter was in place and urine output was good. Airway assessment revealed a short neck, mouth opening of three fingers (Mallampatti Score 2), and a thyromental distance of 7 cm. ECG was normal, and the patient's laboratory investigations were within normal range, which included full blood count, urea and electrolyte levels, and liver function test. Ranitidine 50 mg and metoclopramide 10 mg were administered intravenously.

She was transported to a closed operation room on a trolley in the left lateral position and wore an O_2 face mask. The choice of spinal anaesthesia was discussed with the patient as the first-line procedure. Another 18-gauge cannula was inserted at the dorsum of the right hand. The patient was administered 1 L of intravenous normal saline and ringer lactate as preload and connected to the standard monitoring as per American Society of Anesthesiologists (ASA) guidelines. Her vital signs were a heart rate of 110 beats/min, blood pressure 128/78 mmHg, and O_2 saturation 100% (3 L O_2 face mask). Following preparation and scrubbing of the patient's back, spinal anaesthesia was administered through a long spinal needle (25 gauge) in the left lateral position with 2 mL of Marcaine® Heavy 0.5%. The patient was positioned in a supine position with the left uterus tilted using a wedge under the right buttock. The sensory level was at T4. Vital signs were all within normal range. The abdominal preparation was achieved, and the staff discussed aortocaval compression and syndrome. Furthermore, communication was maintained between the anaesthetist and the patient during the operation. Time of uterus incision from the abdomen took approximately 6 min because surgical access was difficult. After uterine incision, the delivery of the baby was complicated, and 1.5 min passed without delivery of the baby. Communication and reassurance with the patient were maintained. After manual centralisation of the uterus to facilitate the delivery of the baby, the patient became unresponsive and the radial pulse was lost. The left tilt was increased immediately, and the surgeon was notified to deliver the baby in this way because the patient had developed CA. CPR was initiated with chest compression and tracheal intubation was done with cricoid pressure. Pulse and ECG waves returned within approximately 60 sec. The baby was delivered with a total time of 2.5 min from uterus incision. A healthy boy of 3,900 g was delivered, with 1- and 5-min APGAR scores being 7 and 9, respectively. Anaesthesia was maintained with ketamine and atracurium after the patient recovered from CA with 100% O_2. The operation was finished after another 53 minutes with good homeostasis, and the estimated blood loss was 750 mL. The patient was moved to the intensive care unit for post-CA management. She showed stable vital signs, adequate urine output, and satisfactory consciousness level. She was extubated safely one hour after the end of the operation. Her laboratory investigations were all within normal range, which included full blood count, urea and electrolyte levels, coagulation profile, and liver function test, in addition to chest X-ray and ECG. She was moved out of the intensive care unit the next morning, and discharged home on Day 5 with an uneventful postoperative stay and seen with her child 6 weeks later in the postnatal clinic in good condition. Patient consent was obtained for the publication of her case.

DISCUSSION

Although CA is a rare event during pregnancy, it represents a stressful condition as a result of the physiological modifications to the maternal physiology. Thus, resuscitation mandates specific modifications to the standard management. During CPR, a second patient's life needs to be considered in the decision-making process, although the priority is maternal life.[1,2]

Spinal and epidural anaesthesia might be associated with severe bradycardia or hypotension because of the prevention of reflex vasoconstriction in the blocked segment. Therefore, abrupt, severe bradycardia or sinus arrest can occur, but the outcome depends on early management.[3] Compression of the inferior

vena cava through the gravid uterus could result in reducing venous return and right atrial pressure. An acute circulatory collapse, severe enough to mimic haemorrhagic shock, in the supine position may arise and sudden bradycardia may also occur in some cases.[4]

Both Bezold–Jarisch reflex and amniotic fluid embolism might be a cause of CA during CS under general and spinal anaesthesia. Reflex bradycardia is a cardiovascular depression through reflex with vasodilatation and bradycardia. Neuraxial anaesthesia and general anaesthesia could induce sympathectomy, leading to a sudden vasovagal activation, resulting in extreme bradycardia and vasodilatation. Bradycardia could be treated with atropine, ephedrine, or adrenaline. At the same time, significant hypotension following spinal anaesthesia could aggravate the effect of the reflex bradycardia.[5]

Reduction in overweight and obesity are of great importance in leading to a decrease in morbidity and mortality. Morbid obesity no doubt complicates the pregnancy. Both pregnancy and morbid obesity have a pathophysiological change result in multiple manifestations involving different organ systems. Obesity has a deleterious effect on airway difficulty during general anaesthesia as well as haemodynamic instability during neuraxial anaesthesia.[6] Morbidly obese parturient patients have a reduction in respiratory reserve and ventilatory effort.[7] All these factors increase the risk for cardiopulmonary arrest[6,7] and, taken together, pregnancy and severe obesity represent a complex picture and challenging issues for both anaesthetists and obstetricians.[2,8]

Lateral positioning during caesarean delivery is typically considered to be impractical and sometimes full lateral positioning may be essential.[7,9] Prepregnancy obesity and excessive gestational weight gain in parturient patients are associated with a high-risk pregnancy. Pregnancy-induced hypertension, CS, and greater infant size at birth are among these complications.[10] Cluver et al.[11] found that the left lateral tilt was more efficient than the right tilt, while the manual displacement was better than the left lateral tilt. They recommended large studies to support their findings. The use of tilt has a considerable role in reducing the occurrence of inferior vena cava compression during CS and labour. This can be applied effectively by using a 15° tilt during CS and a 30° tilt during labour. Despite this manometer, a minority of women may have susceptibility to inferior vena cava compression.[12,13] The tilt of the patient will cause a significant increase in alveolar-arterial O_2 difference and this should be treated with the administration of a high O_2 concentration.

Ecker et al.[14] reported CA caused by vaginal bleeding during CS in a 43-year-old multigravida woman, under spinal anaesthesia, 20 minutes after delivery of a healthy boy at 36 weeks gestation. She had chest pain, pale lips, apnoea, and rapidly became unresponsive. Successful CPR was performed, and amniotic fluid embolism was reached as the final diagnosis. CA was reported by Farrakh et al.[15] in a 42-year-old, morbidly obese primigravida in the labour room during fetal blood sampling following induction of labour at term due to gestational diabetes and pre-eclampsia. CPR was performed for 3 minutes and then a perimortem CS was performed. She was transferred to theatre for suturing, and a blood transfusion was given accordingly because the total blood loss was 3.5 L using 1:1:1 ratio of packed red blood cells, fresh frozen plasma, and platelets. Amniotic fluid embolism was made as a clinical diagnosis. Both mother and baby showed a good outcome.[15] Banaschak et al. reported a cardiac arrest in a 19-year-old woman during a laparotomy procedure for a persisting uterine haemorrhage after spontaneous delivery of a healthy baby. An air embolism complicated the manual repositioning of the uterine inversion. The autopsy showed extreme gas embolism in both arterial and venous vessels extending from the pelvis to the head.[16] Recognition and initiation of CPR in the lateral position provided a good outcome for mother and baby.[15] The timely perimortem CS delivery improved the chance of maternal survival. Commonly, the 'four-min rule' approach of CPR of pregnant women in the third trimester has been followed: if pulses have not returned within 4 min of the start of resuscitation, a CS should be performed within the next min. However, Benson et al.[17] suggested immediate perimortem CS for better maternal and neonatal chances of survival.[17] A survivor patient in one study showed a median duration from collapse to delivery of 3 min compared with 12 min among those who died.[6]

If the return of spontaneous circulation has not been achieved, additional interventions may include cardiopulmonary bypass and extracorporeal membrane oxygenation Performing a perimortem CS is not a part of training in obstetrics and gynaecology; however, early decision-making and continuation of the operation results in saving both lives. Knowledge gaps are significant in the science of maternal resuscitation. Team training and simulation for readiness for maternal CA should be included during training.[15,18]

CONCLUSION

Anaesthesia and surgery could be complicated by morbid obesity during CS. Correction of fluid status and avoidance of aortocaval compression are important. In the cases of spinal anaesthesia, all resuscitative drugs, equipment, and tools for rapid and good assistant should be ready. Furthermore, maintaining communication with the patient as well as open eyes on standard monitoring is essential. This case demonstrates that the collaboration between the anaesthesia and obstetrical team results in successful outcome management.

References

1. Montufar-Rueda C, Gei A. Cardiac arrest during pregnancy. Clin Obstet Gynecol. 2014;57(4):871-81.
2. Sia AT et al. The ongoing challenges of regional and general anaesthesia in obstetrics. Best Pract Res Clin Obstet Gynaecol. 2010;24(3):303-12.
3. Pollard JB. Cardiac arrest during spinal anesthesia: common mechanisms and strategies for prevention. Anesth Analg. 2001;92(1):252-6.
4. Kinsella SM, Tuckey JP. Perioperative bradycardia and asystole: relationship to vasovagal syncope and the Bezold-Jarisch reflex. Br J Anaesth. 2001;86(6):859-68.
5. van Liempt SW et al. Essentials in cardiac arrest during cesarean section. Clin Pract. 2015;5(1):668.
6. Mhyre JM, Bateman BT. Tipping our CAPS to the UKOSS cardiac arrest in pregnancy study. BJOG. 2017;124(9):1382.
7. Adams JP, Murphy PG. Obesity in anaesthesia and intensive care. Br J Anaesth. 2000;85(1):91-108.
8. Bajwa SJS, Bajwa SK. Anaesthetic challenges and management during pregnancy: strategies revisited. Anesth Essays Res. 2013;7(2):160-7.
9. Campbell TA, Sanson TG. Cardiac arrest and pregnancy. J Emerg Trauma Shock. 2009;2(1):34-42.
10. Li N et al. Maternal prepregnancy body mass index and gestational weight gain on pregnancy outcomes. PLoS One. 2013;8(12):e82310.
11. Cluver C et al. Maternal position during caesarean section for preventing maternal and neonatal complications. Cochrane Database Syst Rev. 2013;3:CD007623.
12. Kinsella SM. Lateral tilt for pregnant women: why 15 degrees? Anaesthesia. 2003;58(9):835-6.
13. Kim YI et al. Successful management of severe aortocaval compression in twin pregnancy. Obstet Gynecol. 1975;46(3):362-4.
14. Ecker JL et al. Case records of the Massachusetts General Hospital. Case 40-2012. A 43-year-old woman with cardiorespiratory arrest after a cesarean section. N Engl J Med. 2012;367(26):2528-36.
15. Farrakh S et al. Cardiac arrest in labour leading to perimortem caesarean section in a woman with a body mass index >46 with a successful outcome. EMJ Repro Health. 2016;2(1):87-9.
16. Banaschak S et al. Fatal postpartum air embolism due to uterine inversion and atonic hemorrhage. Int J Legal Med. 2014;128(1):147-50.
17. Benson MD et al. Maternal collapse: challenging the four-minute rule. EBioMedicine. 2016;6:253-7.
18. Zelop CM et al. Cardiac arrest during pregnancy: ongoing clinical conundrum. Am J Obstet Gynecol. 2018;219(1):52-61.

Smartphone Applications for Reproduction: From Rigorously Validated and Clinically Relevant to Potentially Harmful

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Abstract

Infertility practitioners are increasingly turning to mobile applications (apps) to help improve patient care. Provider-facing apps range from reference to communication tools, to versions of the electronic health record. Some available evidence indicates that mobile apps facilitate patient care by increasing efficiency and accuracy in documentation, information retrieval, and coordination of care.[1] The U.S. Food and Drug Administration (FDA) website currently lists dozens of cleared or approved applications for mobile 'medical device' apps, and reproductive healthcare has also certainly benefitted from this explosion in mobile technology. Reproductive and fertility-focussed apps now aim to treat and diagnose disease, aid clinical decision-making, and manage patient care. Infertility patients may use apps pretreatment to manage lifestyle factors, during treatment to manage medications and calendar appointments, and for message boards where they can share experiences, and seek or offer peer support. Here, the authors review the history of medical health and research apps, current reproduction-specific mobile applications, and discuss the implications of mobile technology for diagnostic point-of-care, clinical research, and patient health management.

INTRODUCTION

Mobile device applications (apps) debuted in 2008, and the world was transformed by these small pieces of software, seemingly overnight. Medical science has been no exception. Over 165,000 health-related apps now improve health, facilitate clinical trials research, and most recently, integrate with sophisticated external hardware, such as microfluidic chambers, to deliver point-of-care diagnostic assays. It has been estimated that at least 500 million smartphone owners use a healthcare app; however, a challenge for any new technology is proving that it meets the accuracy standards of tested, tried, and true methods. New health or research related apps require vigorous validation. This is of great importance to the field of reproduction, where patients are using apps to crowdsource tips on improving fertility and preventing pregnancy, chart cycles by last menstrual period date, time intercourse, track and chart medical information (including cervical fluid, basal body temperature, and fertility medications), and to seek support and share experiences. For this reason, the FDA

issued oversight guidance in 2013 for apps that present a risk to patients if they do not work as intended, and for apps that allow mobile devices to impact the functionality or performance of a traditional medical device. The FDA website lists dozens of cleared or approved applications for mobile medical device apps.[7]

Apple supports the medical community with three products: ResearchKit, HealthKit, and CareKit. These are free, 'open source' resources that allow any app developer to download standard modules from Apple. Generally, CareKit provides tools that lets users regularly track care plans, monitor their progress, and share their insights with care teams. HealthKit allows health and activity data to be tracked and communicated to other apps. ResearchKit allows researchers to conduct clinical studies quickly, cheaply, and easily by collecting survey data through a dedicated study app on the participant's iPhone. These patient-facing apps have harnessed proprietary mobile hardware, (Global Positioning System, accelerometer, gyroscope, touch screen, and microphone) to take and record biometric data.

ResearchKit facilitates Health Insurance Portability and Accountability Act (HIPAA) compliant research projects[3] in disease areas such as asthma, Parkinson's,[4] diabetes, melanoma,[5] and breast cancer, anterior cruciate ligament (ACL) rupture,[6] and cardiovascular disease,[7] but peer-reviewed validation studies have been scarce. The Icahn School of Medicine at Mount Sinai Asthma Mobile Health Study,[8] powered by the ResearchKit-linked iPhone app Asthma Health, has published peer-reviewed survey results in line with existing 'gold standard' research on asthma patients, and rigorous evidence of the app's utility.

Increasingly, smartphone-based medical apps have used sophisticated external hardware add-ons to perform everything from enzyme-linked immunosorbent assay (ELISA) assays,[9,10] to ultrasonography,[11] parasitic microscopy,[12] and quantum dot barcoding for rapid viral detection.[13] However, these devices and apps tend to be targeted to resource-poor geographical locations, designed to be deployed to, and have specimens collected by, laboratory technicians, with the results intended to be read and interpreted by healthcare professionals before communication to the patient.

On 10th September 2019, Apple made another major announcement that extended its support of the medical research community. They announced three 'unprecedented' medical studies, in partnership with leading academic and research institutions that will be available on the new Research app, which claim to "democratizes how medical research is conducted by bringing together academic medical institutions, healthcare organizations and the Apple products customers already make a part of their everyday life." Along with a comprehensive heart/movement study and a hearing study, Apple announced a major fertility initiative.

From Apple's press release:

"Apple Women's Health Study: In partnership with Harvard T.H. Chan School of Public Health and the NIH's National Institute of Environmental Health Sciences (NIEHS), Apple has initiated the first long-term study of this scale focused on menstrual cycles and gynecological conditions. This study will inform screening and risk assessment of conditions like polycystic ovary syndrome (PCOS), infertility, osteoporosis, pregnancy and menopausal transition."[14]

The last study is of particular interest to the field of reproduction, where the use of apps is a burgeoning trend. There are currently over 100 fertility awareness mobile apps[15] with more than 200 million downloads, being used for contraception and pregnancy planning. Excitingly, fertility awareness apps are just scratching the surface of what is possible in reproductive mobile healthcare.

MOBILE APPLICATIONS FOR REPRODUCTION

Reproduction apps touch every aspect of the field: diagnostics, clinical research, patient communication and education, embryology laboratory quality control, and medical education. Specific reproduction apps are reviewed in the following section (Table 1) .

Diagnostic/Point-of-Care

In one of the first examples of its kind for reproduction, a sophisticated external micro-fluidics hardware add-on has been paired with a consumer-facing (no clinical background needed)

application for diagnostic semen analysis.[16] The results were published in a rigorously validated and peer-reviewed study using standard World Health Organization (WHO) criteria[3] for sperm concentration, motility, total sperm count, total motile sperm count, and linear and curvilinear velocities and benchmarked results against gold standard computer-assisted semen analyses (CASA). Additionally, the usability and simplicity of the app was evaluated by recruiting untrained users to participate in a double-blinded evaluation of semen analysis using the smartphone-based platform and CASA. This application was not commercialised at the time of writing this review.

Subsequently, a similar commercially available application called "YO" was FDA approved for motile sperm testing and concentration. It has a cut off value above and below a 6 million per mL, therefore it is not recommended for specialised tests, like vasectomy confirmation. The YO device demonstrated good correlation and good to moderate agreement with an automated semen analyser. The precision among the YO phone devices was lower (16.0%) than the manufacturer's claim of ≤20.0%.[17]

Clinical Reproduction Research

Pregnant women have been under-represented in research studies, resulting in dangerous deficiencies in evidence-based guidance for treatment. To that end, Topol et al.[18] implemented the first pregnancy research app for large-scale collection of survey and sensor-generated data to improve our understanding of factors that promote a healthy pregnancy (for both the mother and developing fetus). They enrolled 2,058 demographically diverse pregnant women from all 50 states, fairly representing USA population averages. They summarised the findings from 14,045 individual surveys and 107,102 total daily measurements of sleep, activity, blood pressure, and heart rate, demonstrating the potential for a smartphone-based research platform to capture an array of longitudinal, objective, and subjective participant-generated data from an under-represented and diverse population of pregnant women.

Table 1: Uses of mobile applications in reproduction.

Mobile apps	Diagnostic/point of care	Clinical research	Infertility patient	Quality control	Medical education
YO Sperm Test	Sperm motility and concentration				
Premom	LH test				
Healthy Pregnancy		Healthy pregnancy characteristics			
Natural Cycles		Stages of ovulation			
SART			IVF clinics and treatment options		
IVF Planner			Medications, appointments, symptoms		
IVFqc				Instruments	
ART Compass				Staff competency	
Embryo App					Developmental biology

Selected mobile applications in several use categories.

ART: assisted reproductive technology; LH: luteinising hormone; SART: The Society for Assisted Reproductive Technology.

Bull et al.[19] analysed over half a million ovulation cycles worth of data collected via the FDA-approved 'Natural Cycles' app to rewrite understanding of the key stages of ovulation. They showed that few women have the textbook 28-day cycle, with some experiencing very short or very long cycles. The findings show an average cycle length is 29.3 days and only around 13% of cycles are 28 days in length. Across the study, only 65% of women had cycles that lasted between 25 and 30 days. The Natural Cycles app claims to be useful as a hormone-free method of birth control, studies have demonstrated a 'typical use' failure rate over 13 menstrual cycles of 8.3%.[20]

Infertility Patient Awareness and Education

The Society for Assisted Reproductive Technology (SART) recently launched the SART Mobile App.[21] The app includes a calculator that offers personalised information based on years of national research data and millions of patients and treatments. IVF patients can submit their individual information and receive feedback for various treatment options. They can find and speak with a fertility clinic, schedule clinic appointments, and even manage treatment information. The app also includes a pregnancy wheel and a section for IVF news and information.

An additional 25 apps out of 140 reviewed (17.9%) contain information or functions specifically related to infertility or its management.[22] High quality infertility applications were noted as allowing users to track fertility medications, symptoms, and results. Additional features include reminders of fertility doctor appointments and when to administer fertility medication, results tracking (including blood type information, sperm counts, and blood levels), notes section for tracking of issues for later reference, and ability to track symptoms.

IVF Laboratory Digital Quality Control

There are at least 200 known variables that can impact IVF outcome.[23] The potential linking of quality control performance to enhanced patient care and outcomes using mobile app technology is intriguing. IVFqc recently released an app called Reflections™ that allows customisation of quality control for any laboratory instrument and associated parameters.[24] The app is accessible from any internet-connected device (computer, tablet, or smartphone of any make) and allows for real-time instrument fluctuation tracking and detailed reporting.[25] They recently reported quality control data from 36 clinics across 12 countries.[26]

In the USA, competency of personnel responsible for testing in an IVF lab is required to be evaluated at least semiannually during the first year the individual tests patient specimens, and at least annually thereafter. Competency assessment must be performed for testing personnel for each test that the individual is approved by the laboratory director to perform. Curchoe et al.[27] created a HIPAA compliant mobile application[28] to assess the clinical decision-making of ART laboratory staff for more than 80 common andrology and embryology procedures, track new staff competency during employee on-boarding and annually thereafter, and provide real time in-cycle statistics for staff-related IVF cycle parameters.

Eggschain[29] is a blockchain-based mobile app that allows women to create a digital, trackable identity using blockchain technology for their frozen eggs and embryos. Eggschain aims to utilise the "immutable and secure" nature of blockchain technology to help women to indisputably prove that frozen eggs and embryos are their own. To date, there have been no abstracts or primary literature published evaluating these claims.[29]

Embryologist Education Applications

The Carnegie Collection of Embryology, housed at the National Museum of Health and Medicine (NMHM), Silver Spring, Maryland, USA, is used by embryologists worldwide to define normal human embryo development. The Embryo App includes films on fertilisation and IVF, a pregnancy calculator, and a lab manual section. The lab manual presents photographs, 3D reconstructions, animations, reference labels, and information on the early stages (1–23) of embryonic development. The application links to social networks and to extended resources from the National Library of Medicine, Bethesda, Maryland, USA; Louisiana State University, Baton Rouge, Louisiana, USA; NMHM and other institutions.[30]

DISCUSSION

Mobile apps are increasingly used in reproductive healthcare to promote wellness, treat, diagnose, aid clinical decision-making, manage patient care, and collect data for medical research. Mobile app technology has many advantages: HIPAA compliance, rapid data collection and real time reporting, real-time analysis, management and distribution of multi-media files, and the ability to utilise hardware add-ons or proprietary device hardware features, and to collect biometric data to ensure the identity of the tester. However, questions remain about the clinical validity and utility of these new mobile tools for reproductive healthcare. Known limitations of mobile apps include: lack of evidence of clinical effectiveness, lack of integration with the healthcare delivery systems, lack of formal evaluation and review, and potential threats to safety and privacy.[31]

Quality assessment methodologies and tools for mobile apps have been adapted from assessments for other digital technologies, such as websites. Nouri et al.[32] reviewed currently used models, codes, and scales for assessment of mobile health-related apps. Important assessment criteria included: accuracy, information quality, and security among others. Menstrual tracking apps have been consistently assessed for their functionality and accuracy. In 2016, Moglia et al.[33] scored 108 menstrual tracking apps, and their primary criterion for ongoing inclusion was accuracy. They concluded "Most free smartphone menstrual cycle tracking apps for patient use are inaccurate. Few cite medical literature or health professional involvement."[33] Updating this analysis in 2019, Zwingerman et al.[22] identified 140 menstrual tracking apps, with a low overall app quality score of 32%, and a further 31 apps (22.1%) with serious inaccuracies in content, tools, or both. When 218 menstrual tracking apps were assessed in 2016 for their use in preventing unintended pregnancy, over 40% were found to not mention any modern contraceptive methods at all.[34] This systematic review by Mangone et al.[34] found that very few fertility awareness apps have clinically relevant, evidence-based usefulness, and many of them may even increase the likelihood of unintended pregnancy due to the low effectiveness of the contraceptive methods promoted.[34] For this reason, the American College of Obstetricians and Gynecologists (ACOG) only advocates use of mobile applications to track menstrual cycles, not as a primary tool to prevent or achieve pregnancy.[33]

The Natural Cycles mobile app investigated by Bull et al.[18] has a 'typical use' failure rate of preventing pregnancy up to 8.3% of the time.[19] In some cases, the failure of the app to prevent pregnancy has resulted in pregnancy-terminations, generating lawsuits in their wake.[35] The findings of Bull et al. have significant implications for fertility awareness apps that use 'ideal' cycle calendars to generically calculate fertile windows to either plan or prevent a pregnancy. Additionally, there have been major HIPAA compliance concerns with some fertility tracking apps. These applications often ask for intimate details: sexual activity, history of abortions, cervical mucus consistency, orgasm frequency, and preferred sex positions. It was recently reported that the Glow (a pregnancy planning app) was plagued by a series of security flaws, exposing sensitive information to anyone who cared to look. It was characterised as a "jackpot for stalkers."[36] They have since added a new section to their website, inviting hackers to "research" security flaws and responsibly report them.

Without question, laboratory quality control and assurance must be performed routinely in an IVF lab. While the embryologist's role in achieving and contributing to quality[37] through safety in the assisted reproduction lab is obvious; appropriate levels of monitoring, what to monitor, and the best ways to monitor it are surprisingly unclear. Until just recently, the anatomy of a liquid nitrogen dewar failure,[38] how a storage vessel behaves when the vacuum is breached, was unknown, and recent investigations have also quantified major differences in instrument monitoring practices worldwide.[26] Staff competency is a crucial component of the IVF laboratory's quality management system because it directly impacts clinical outcomes. Embryologists must be competent to make several clinical decisions that can affect cycle outcomes. Certain IVF key performance indicators[39] are used to continuously monitor and assess culture conditions.

Digital and 'cloud based' solutions to discover malfunctioning instruments and environments have been best practice tools for over 20 years in other industries such as aerospace, automotive,

and manufacturing. It seems as though the time of digital quality control is long overdue for the IVF lab, and that IVFqc and ART Compass represent a new paradigm, but future publications detailing how the measured parameters relate to clinical outcomes will help to further advance the field of IVF lab quality control.

Cryopreservation of reproductive tissues was once an adjunctive procedure, but in contemporary IVF labs it enables preimplantation genetic diagnosis after blastocyst biopsy, deferred transfer to a more favourable uterine environment, and fertility preservation. Chain of custody for reproductive tissues is related to both staff competency and quality control, with cryopreservation and storage of human embryos and gametes emerging as a clear subspecialty of the assisted reproductive technologies industry.[40] New technologies, including a mobile app that promises to help solve chain of custody issues, such as pairing the wrong gametes together or the wrong embryo with the intended parent, are needed. The Eggschain mobile app, while promising, is marketing a 'clear chain of custody for decisions by-laws, contracts, estate plans.' It is unclear at the time of writing if the technology could fulfill these promises, or if it has been tested in a court of law.

CONCLUSION

The challenge for these new technologies is to prove that they meet the accuracy standards of tested, tried, and true methods. New health or research-related apps that have the potential to impact patients, curate very sensitive health data, and or impact patient care require vigorous validation.

References

1. Ventola CL. Mobile devices and apps for health care professionals: uses and benefits. P T. 2014;39(5):356-64.
2. US Food and Drug Administration (FDA). Examples of Pre-Market Submissions that Include MMAs Cleared or Approved by the FDA. 2018. Available at: www.fda.gov/medical-devices/device-software-functions-including-mobile-medical-applications/examples-premarket-submissions-include-mmas-cleared-or-approved-fda. Last accessed: 06.04.2020.
3. Rosa C et al. Using e-technologies in clinical trials. Contemp Clin Trials, 2015;45(PtA):41-54.
4. Bot B et al. The mPower study, Parkinson disease mobile data collected using ResearchKit. Sci Data. 2016;3:160011.
5. Webster D et al. The Mole Mapper Study, mobile phone skin imaging and melanoma risk data collected using ResearchKit. Sci Data. 2017;4:170005.
6. Zens M et al. "Back on Track": a mobile app observational study using Apple's ResearchKit framework. JMIR Mhealth Uhealth. 2017;5(2):e23.
7. McConnell M et al. Feasibility of obtaining measures of lifestyle from a smartphone app: the MyHeart Counts cardiovascular health study. JAMA Cardiol. 2017;2(1):67-76.
8. Chan YF et al. The asthma mobile health study, a large-scale clinical observational study using ResearchKit. Nat Biotechnol. 2017;35(4):354-62.
9. Laksanasopin T et al. A smartphone dongle for diagnosis of infectious diseases at the point of care. Sci Transl Med. 2015;7(273):273re1.
10. Berg B et al. Cellphone-based hand-held microplate reader for point-of-care testing of enzyme-linked immunosorbent assays. ACS Nano. 2015;9(8):7857-66.
11. Wojtczak J, Bonadonna P. Pocket mobile smartphone system for the point-of-care submandibular ultrasonography. Am J Emerg Med. 2013;31(3):573-7.
12. D'Ambrosio M et al Point-of-care quantification of blood-borne filarial parasites with a mobile phone microscope. Sci Transl Med. 2015;7(286):286re4.
13. Ming K et al. Integrated quantum dot barcode smartphone optical device for wireless multiplexed diagnosis of infected patients. ACS Nano. 2015;9(3):3060-74.
14. Apple Inc. Apple announces three groundbreaking health studies. 2019. Available from: www.apple.com/newsroom/2019/09/apple-announces-three-groundbreaking-health-studies/ Last accessed: 07.04.2020.
15. Dreaper J. Women warned about booming market in period tracker apps. 2016. Available at: www.bbc.co.uk/news/health-37013217. Last accessed: 20.03.2020.
16. Kanakasabapathy MK et al. An automated smartphone-based diagnostic assay for point-of-care semen analysis. Sci Transl Med. 2017;9(382).
17. Agarwal A et al. Home sperm testing device versus laboratory sperm quality analyzer: comparison of motile sperm concentration. Fertil Steril. 2018;110(7):1277-84.
18. Radin J et al. The healthy pregnancy research program: transforming pregnancy research through a ResearchKit app. NPJ Digit Med. 2018; 1:45.
19. Bull J et al. Real-world menstrual cycle characteristics of more than 600,000 menstrual cycles. NPJ Digit Med. 2019;2:83.
20. Berglund Scherwitzl E et al. Perfect-use and typical-use Pearl Index of a contraceptive mobile app. Contraception. 2017;96(6):420-5.
21. Society for Assisted Reproductive Technology, Apple Store. SART mobile: the official SART app for IVF. 2020. Available from: https://apps.apple.com/us/app/sart-mobile/id1370121034. Last accessed: 20.03.2020.
22. Zwingerman R et al. A critical appraisal of fertility and menstrual tracking apps for the iPhone. J Obstet Gynaecol Can. 2019. [ePub ahead of print].
23. Pool T et al. Human embryo culture media comparisons. Methods Mol Biol. 2012;912:367-86.

24. Althea Science Inc. IVFqc. 2020. Avaiable at: www.ivfqc.com. Last accessed: 20.03.2020.
25. Fiser S et al. IVF data flow: from trash bins to clipboards to EMR to smart apps. Fertility. 2016;19:24-6.
26. Palmer G et al. Comparison of 36 assisted reproduction laboratories monitoring environmental conditions and instrument parameters using the same quality-control application. Reprod Biomed Online. 2019;39(1):63-74.
27. Curchoe C et al. Development of a mobile competency assessment platform for IVF laboratory quality managment systems. Abstract Foundation for Reproductive Medicine Annual Conference, 15-18 November, 2018.
28. ART Compass. ART Compass. Available at: www.artcompass.io. Last accessed: 20.03.2020.
29. Alice. Eggschain is empowering women who are choosing to freeze their eggs. 2018. Available at: https://medium.com/successful-entrepreneurs-role-breakers/wei-escala-eggschain-successful-women-entrepreneurs-68e09ca98659. Last accessed: 20.03.2020.
30. Haseltine F et al. The Mobile Embryo Application. FASEB J. 2011;25(Suppl 1):lb12.
31. Carroll J et al. Who uses mobile phone health apps and does use matter? a secondary data analytics approach. J Med Internet Res. 2017;19(4):e125.
32. Nouri R et al. Criteria for assessing the quality of mHealth apps: a systematic review. J Am Med Inform Assoc. 2018;25(8):1089-98.
33. Moglia M et al. Evaluation of smartphone menstrual cycle tracking applications using an adapted APPLICATIONS scoring system. Obstet Gynecol. 2016;127(6):1153-60.
34. Mangone E et al. Mobile phone apps for the prevention of unintended pregnancy: a systematic review and content analysis. JMIR Mhealth Uhealth. 2016;4(1):e6.
35. Global News. Natural Cycles contraceptive app blamed for 37 unwanted pregnancies. 2018. Available at: https://globalnews.ca/news/3967648/contaceptive-app-unwanted-pregnancy/. Last accessed: 20.03.2020.
36. Weill K. This fertility app is a jackpot for stalkers. 2017. Available at: https://www.thedailybeast.com/this-fertility-app-is-a-jackpot-for-stalkers. Last accessed: 20.03.2020.
37. Go K. 'By the work, one knows the workman': the practice and profession of the embryologist and its translation to quality in the embryology laboratory. Reprod Biomed Online. 2015. 31(4):449-58.
38. Pomeroy K et al. Cryostorage tank failures: temperature and volume loss over time after induced failure by removal of insulative vacuum. J Assist Reprod Genet. 2019;36(11):2271-8.
39. ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine. The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators. Reprod Biomed Online. 2017;35(5):494-510.
40. Go K. A subspecialty of the assisted reproductive technologies: cryogenic inventory maintenance. J Assist Reprod Genet. 2019;36(1):15-17.