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ESHRE 2021

EDITOR'S PICK

The Use of Frozen Embryo Transfer and the Development of Pregnancy-Induced Hypertension: A Literature Review

INTERVIEW

Interview with Jennifer Hall

Contents

"Prepare yourselves for the latest reproductive medicine updates, including a comprehensive review of the 37th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE)."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

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Front cover and contents photograph: National Palace of Pena in Sintra, near Lisbon, Portugal, home of ESHRE 2021. © yasonya / 123rf.com

EMJ Repro Health 7.1

Welcome

Welcome to this issue of *EMJ Reproductive Health*! Prepare yourselves for the latest reproductive medicine updates, including a comprehensive review of the 37th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE). Included are fascinating abstract summaries of the latest, hot topic research in the field. Read on for peer-reviewed articles and an exclusive interview with an industry expert.

Despite a move to the virtual, the ESHRE 2021 was nothing short of excellent in its seamless delivery of a variety of subjects over six fully loaded days of scientific sessions. In our congress review, we highlight a discussion held by famous psychologist Massimo Polidoro, who touched upon the ongoing conspiracy theories associated with the COVID-19 pandemic and its dangerous consequences. Abstract summaries from this leading congress span multiple research areas, including 'Hypnofertility for Reducing Stress and Increasing Fertility Preparedness' and 'Reproductive Performance of Females With and Without Intrauterine Adhesions Diagnosis.'

In this eJournal we have a selection of peer-reviewed articles that offer cutting-edge research, including diagnostic criteria and treatment modalities of ectopic pregnancies and an examination of diagnostic options and classification systems available for endometriosis. The Editor's Pick for this journal is 'The Use of Frozen Embryo Transfer and the Development of Pregnancy-Induced Hypertension: A Literature Review' by Agbabiaka and D'Angelo.

We had the pleasure of interviewing Jennifer Hall, Clinical Associate Professor and NIHR Advanced Fellow at University College London Institute for Women's Health. Be sure to read this exclusive interview as we sat down to discuss her role and motivations in the practice of reproductive medicine and future research topics in the field.

All that is left is for me to thank the Editorial Board, authors, and interviewee for their continued support. It is through these incredible contributions that we are able to provide this high-quality content and bring the latest research and industry developments to you. Thank you for choosing EMJ to be the go-to place for healthcare professionals and we look forward to seeing you all in Milan for ESHRE 2022.

Spencer Gore

Chief Executive Officer, EMG-Health

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Foreword

Dear Colleagues,

I would like to give you a warm introduction to the EMJ Reproductive Health journal, containing the latest insights, peer-reviewed articles, and informative highlights from this year's European Society of Human Reproduction and Embryology (ESHRE) Congress. This eJournal covers a broad range of topics, from the effects of using frozen embryos to assessing the impact of COVID-19 on in vitro fertilisation (IVF) treatment.

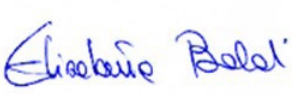
The 2021 virtual ESHRE Congress had fascinating presentations on the most up-to-date research in IVF treatment, and the congress review highlights section includes our pick of the most interesting research, including how the use of artificial intelligence and computer vision remove the need for cell biopsy in testing embryos.

This publication has the most recent peer-reviewed articles from several leading authors. Abdelazim et al. discuss the diagnostic criteria and treatment modalities of ectopic pregnancies in a fascinating literature review. A case report by Leal et al. explains a successful transverse uterine incision in a 23-year-old patient. This exciting collection of reviews and latest insights covers a range of topics in reproductive health, making it a very informative and insightful read.

The Editor's Pick for this publication is the interesting review by Agbabiaka and D'Angelo, who examine the use of frozen embryo transfers (FET) and the development of pregnancy-induced hypertension. The use of frozen embryos in assisted reproduction was successfully introduced some years ago, with good outcomes in terms of pregnancy rate and live birth. However, whether pregnancy-induced complications such as hypertension and pre-eclampsia are different respect to fresh transfers is less clear. The authors, after reviewing the studies that report the incidence of hypertension and pre-eclampsia after FET, evidence a possible relation between FET and hypertension, with possible implications for clinical practice.

We really hope you enjoy reading our EMJ Reproductive Health journal.

Kind Regards,



Elisabetta Baldi

Associate Professor of Clinical Pathology, University of Florence, Italy; Responsible of the Laboratory of Semen Analysis and Semen Bank of the University Hospital of Careggi, Florence, Italy; Responsible of the Laboratory of Assisted Reproduction of the University Hospital of Careggi, Florence, Italy.

Congress Review

Review of the 37th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE)

Location: ESHRE 2021

Date: 26th June –1st July 2021

Citation: EMJ Repro Health. 2021;7[1]:11-22. Congress Review.

VIRTUAL has become the main approach for communicating worldwide and we have all had to flexibly adjust due to the ongoing COVID-19 pandemic. This was the second year that the European Society of Human Reproduction and Embryology (ESHRE) Congress was held as an online meeting instead of the usual face-to-face. Cristina Magli, who has been serving as the ESHRE Chair for 2 years, opened the ceremony by welcoming all the participants to the annual virtual meeting. However, she stated that this will be her last year serving in this position and her successor for the following years will be Carlos Calhaz-Jorge, Human Reproduction Unit, Faculdade de Medicina da Universidade de Lisboa, Portugal, who has also been an active member within the society for several years.

One of the highlights of the opening ceremony was the special appearance of Massimo Polidoro, a famous psychologist who was invited to discuss the ongoing 'fake news' and conspiracy theories associated with the COVID-19 pandemic. According to Magli, during the COVID-19 pandemic a group of individuals hesitant to receiving the COVID-19 vaccine had started a rumour about the vaccine causing infertility in females, which she confirmed was entirely fabricated. The ESHRE society have an active COVID-19 working group that was established on 25th March 2019 in response to the COVID-19 pandemic. This group has published nine statements on COVID-19 and vaccination and effects on assisted reproductive technology and pregnancy.

Polidoro started his session by explaining how we live in a polarised world populated with different perceptions, beliefs, and most certainly conspiracy theories. He continued by stating how fake news and conspiracy theories during the COVID-19 pandemic have been particularly dangerous this the past year. Ridiculous claims such as that eating garlic and injecting disinfectant into the blood stream could cure the virus have been particularly harmful. "A common trait of these such ideas is the denial of reality," said Polidoro. The rejection of

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facts and suggestions of alternative views show that, unfortunately, certain individuals may have trouble accepting the complexities of the present times. The boundaries between the reality and fantasy are often blurred and it is therefore sometimes difficult to dissociate. According to Polidoro, fake news can be spread more rapidly due to the internet. The psychologist stated that our perceptions and reasoning can easily be misled due to the constant battle between our rationalised fear and that based off our instincts. Generally, when the brain processes information opinions are formed quickly and more instinctively; however, an in-depth assessment of this information in the brain takes longer and requires more energy. As humans, we tend to seek confirmation for our beliefs even though they may be wrong; this is referred to by psychologists as cognitive dissonance. Polidoro suggested that we should all think like scientists when information is provided to us especially via the web: cultivate scepticism, finding the source of information and identifying any conflicts of interest and credibility, verifying information before sharing it, and recognising our limitations by being aware of our cognitive biases. Finally, Polidoro stated that correlation is not similar to causation, and further explained that the fact that two phenomena occur simultaneously does not mean that one is the cause of the other. He concluded the session by stating, "always keep an open mind when faced with new discoveries or information."

The ESHRE 2021 meeting was, for the first time, extended from 4 to 6 days. The first 2 days covered several insightful pre-congress courses and the remaining 4 days were solely dedicated to a scientific programme featuring plenary, keynote, and industry-sponsored sessions. There was an all-time record membership of 10,731, which was an increase of 13% compared to the previous year. There were 774 ePosters and 1,312 abstracts. Awards in this year's ESHRE congress covered five presentations, selected and rewarded with a prize of 2,000 EUR. Cheow Yuen Tan, Robinson Research Institute, Department of Obstetrics and Gynaecology, The University of Adelaide, Australia, was awarded the best oral presentation on a basic science topic. Maria Schubert, Centre of Reproductive Medicine and Andrology (CeRA), Department of Clinical and Surgical Andrology, University Münster, Germany, was awarded the best oral presentation on a clinical science topic. Chang Liu, Reproductive Medicine Center, Nanjing University Medical School, China was awarded the prize for best poster presentation on a basic science topic. Marine Grellet-Grün, Department of Reproductive Biology, Centre Hospitalier Universitaire de Reims, France was awarded best poster presentation on a clinical science topic. Bieke Bekaert, Department of Human structure and Repair, Ghent University, Belgium was selected for an exchange educational travel grant award, to present the data of an oral presentation from the annual meeting of the Fertility Society of Australia (FSA). Paulien Pijpops, Department for Development and Regeneration, Leuven University, Belgium, was awarded the nurse award for best oral presentation.

The ESHRE Chair concluded the meeting by thanking all the participants and presenters. She stated that ESHRE's 2022 annual meeting will take place in Milan and will be the Society's first hybrid event. ■

ESHRE 2021 REVIEWED →

Increased Perinatal Health Risks with Use of Non-Prescription Painkillers

MONUMENTAL evidence has been collated from a study spanning 30 years, suggesting women who use over-the-counter painkillers during pregnancy are 1.5-times more likely to have a baby with health issues. Study of this relationship included focus on preterm delivery, stillbirth or neonatal death, physical defects, and a host of other outcomes, all of which were less frequent in mothers not taking the aforementioned drugs. The emerging evidence, from the University of Aberdeen, Scotland, has impact upon the healthcare guidance for pregnant women; Aikaterini Zafeiri, the lead investigator in the current study, who shared the findings at ESHRE 2021, suggested this information "should be reassessed" in line with their findings.

Utilising data between 1985 and 2015 from the Aberdeen Maternity and Neonatal Databank, the medical notes for a total of 151,141 singleton pregnancies were analysed for the consumption of five painkillers: paracetamol, aspirin, diclofenac, naproxen, and ibuprofen. Results demonstrated 3 out of 10 (29%) women took unprescribed analgesics during pregnancy, a figure which increased dramatically to reach 60% in the last 7 years of the study period. These results imply that use is growing at an alarming rate, especially when it is observed that 84% of women reported use during the first 12 weeks after conception. Adjusted odds ratios for the health risks of use of at least one of the five drugs were neural tube defects: 1.64%; admitted to a neonatal unit: 1.57%; neonatal death: 1.56%; premature delivery (before 37 weeks): 1.5%; baby's condition at birth scoring <7 at 5 mins (on appearance, pulse, grimace, activity and respiration; APGAR scale): 1.48%; stillbirth: 1.33%; birth weight <2.5 kg: 1.28%; hypospadias: 1.27%; APGAR score <7 at 1 min: 1.18%; and birth weight >4 kg: 1.09%. Paracetamol use alone was not significantly associated with high birth weight, neural tube defects, or hypospadias; meanwhile, diclofenac consumption was associated with significantly decreased odds of stillbirth, potentially explained by its anti-inflammatory profile.

This large longitudinal study raises great clinical awareness when it is considered that 30–80% of women use painkillers globally to alleviate pain and other unfavourable symptoms during pregnancy. Undoubtedly, the findings will improve and clarify the dissemination of information about the safe use of drugs during gestation, which at the moment varies widely, with some considered safe and others not. Zafeiri highlighted these safety concerns, stating that the ease of access to painkillers is exaggerated by incorrect information found on the internet: "This is especially when misinformed or partially-informed self-medication decisions are taken during pregnancy". ■

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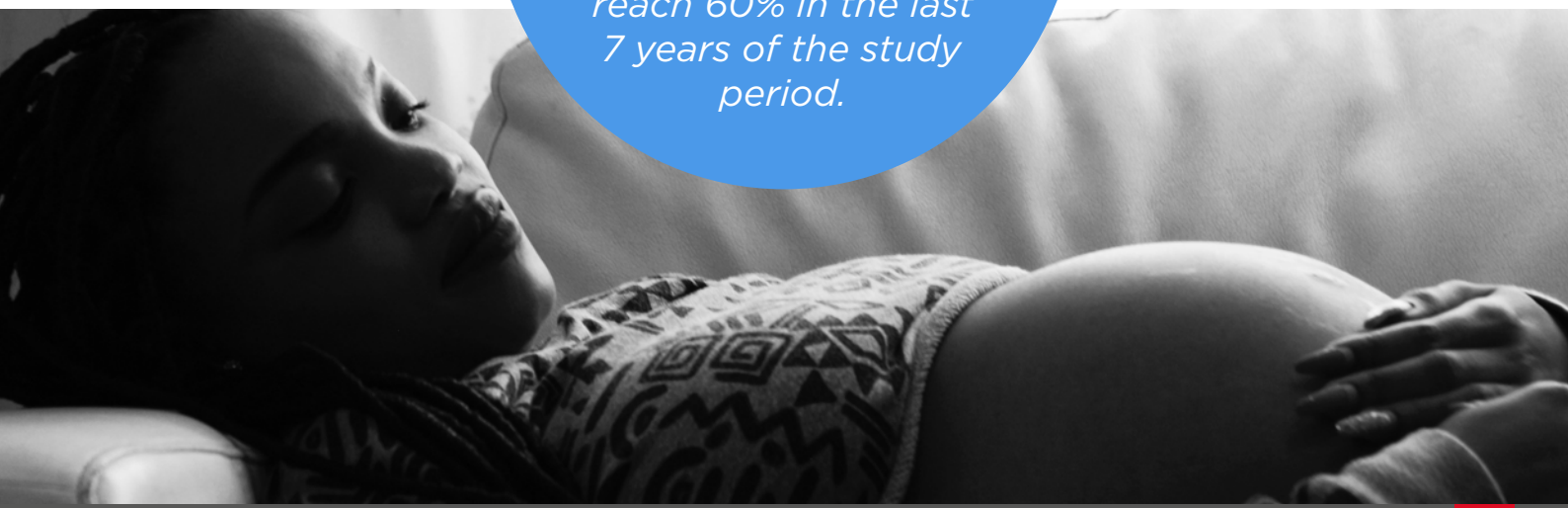


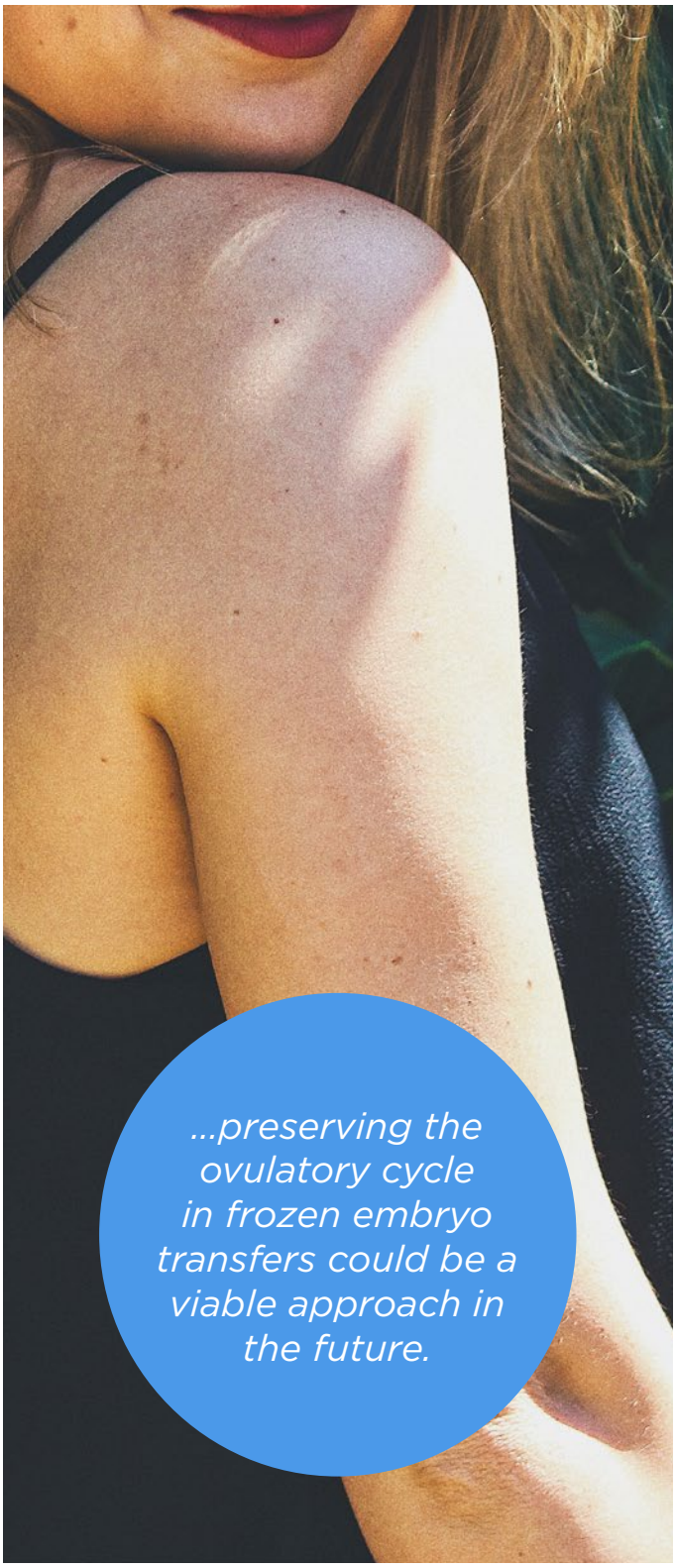
Embryo Freezing in IVF Increases Blood Pressure Risk in Pregnancy

SUCCESSFUL embryo freezing started in the 1980s, and patients can now choose to freeze their healthy embryos to have children later in life. In the past, embryo freezing has not revealed any worrying side effects. However, a recent study, shared at ESHRE 2021, found a potential link between embryo freezing for *in vitro* fertilisation (IVF) and blood pressure problems in pregnancy when the uterus is ready for hormone replacement therapy.

A 2013–2018 study in France involving a large cohort of 70,000 pregnancies from an IVF database, delivered after 22 weeks gestation, analysed the effects of embryo freezing in pregnancy. The patients were divided into three groups: n=10,373 pregnancies from frozen embryo transfer with hormone replacement therapy, n=9,500 pregnancies from frozen embryo transfer in a natural ovulatory cycle, and finally n=48,152 conventional fresh transfers.

The results revealed that there was a significantly higher risk of high blood pressure and hypertension in pregnancies with frozen embryo transfer with hormone replacement therapy (5.3%) compared to frozen embryo transfer in a natural ovulatory cycle (2.3%) and fresh embryo transfers (2.4%). Slyvie Epelboin, from the Bichat–Claude Bernard Hospital, Paris, France, presented these results at the congress and explained that the data indicated two vital considerations in IVF. Firstly, the possibility of high blood pressure and hypertension in frozen embryo transfer in hormone replacement therapy and secondly, that the corpus luteum may have a protective effect. Epelboin infers that hormone replacement therapy affects the formation of the corpus luteum and therefore no longer protects individuals from vascular pathologies.

Furthermore, it is important to consider whether the benefits of embryo freezing with IVF outweigh the risks; results show that currently, it does. Embryo freezing with IVF has a lower chance of hyperstimulation, indicating that this method has its safety benefits. Scientists concluded that there is a higher risk of vascular pathologies in pregnancies with frozen embryo transfer and hormone replacement therapies in comparison to preserved ovulation and using fresh embryos. The study summary for ESHRE 2021 added that preserving the ovulatory cycle in frozen embryo transfers could be a viable approach in the future. ■



Double Stimulation Approach to Improve IVF Response

IN 1978 the world's first baby was born by *in vitro* fertilisation (IVF). This treatment dates to over half a century ago. Since then, IVF has undergone further development, and new technology has helped improve responses. The traditional approach of IVF treatment involves a single dose in a single cycle; if this approach fails, then the patient may be offered to have another round of IVF treatment. Clinical guidelines suggest that increasing the drug dose has little benefit in improving response. Poor responses in particular patient groups remain a key challenge.

Irrespective of the clinical guidelines, a randomised trial, shared in a press release at ESHRE 2021, evaluated the efficacy of two cycles of ovarian stimulation and two collections of eggs. This double stimulation approach was coined as 'DuoStim'. Researchers recruited 80 patients, aged >38 years, undergoing IVF treatment. Patients who took part in the trial had had a low response to IVF in the past; this was confirmed by ovarian reserve tests and reproductive history.

To compare the efficacy of a double stimulation approach with the conventional single cycle, patients were randomised to receive either DuoStim or two separate cycles of stimulation. Interestingly, results showed that DuoStim was just as effective as the two separate cycles, with the added benefit that DuoStim is faster. The results also showed a significant difference in the time it took to develop a normal embryo for transfer. In the DuoStim group, this occurred on average 23 days after stimulation, whereas in the other group it took 21 days longer.

Another added benefit of DuoStim is that fewer patients dropped out of this treatment due to the shorter time required. Maria Cerrillo Martinez explained how this new approach might be beneficial to patients with limited time available, including patients with cancer and older patients.

Cerillo concluded: "It may be a good alternative in poor responders, in fertility preservation patients with time constraints, or even in egg donors, whose aim is to maximise the number of eggs retrieved in a single treatment." ■

IVF Success Rates Is Due to Late-stage Embryo Transfer and Embryo Freezing

...the blastocyst transfer in IVF, whereby embryos are cultured for 5–6 days and then transferred after freezing, was noted to be an important contributor to the improved birth rates over time.

SUCCESSFUL *in vitro* fertilisation (IVF) rates could be due to the implementation of two widely used techniques, late-stage embryo transfer and embryo freezing, following the results for a large cohort observation study carried out in from the national IVF registry of Sweden. The study involved approximately 125,000 treatments that took place between 2007 and 2017, and was presented on 30th June 2021 at ESHRE 2021 by Zoha Saket, Sahlgrenska University Hospital, Gothenburg, Sweden.

According to the national data analysed, the accumulation of live birth rates increased from 27% in 2007 to 36% in 2017 following each egg retrieval cycle. Previous studies showed that the transfer of blastocysts was linked with higher birth rates compared to cleavage-stage embryos. Additionally, the rise in the overall live birth rate was found to be independent of age, the number of eggs collected, and the number of preceding IVF live births, which suggested that the treatment methodology was the main cause of the IVF live birth success rates.

Saket stated that the blastocyst transfer in IVF, whereby embryos were cultured for 5–6 days and then transferred after freezing, was noted to be an important contributor to the improved birth rates over time. Currently, the majority of IVF registries state that approximately 75% of all embryo transfers happen during the blastocyst stage. This transfer is similar to physiological processes of natural pregnancy as the embryo is implanted in the uterus at the same blastocyte stage. A revolutionary rapid-freezing technology called vitrification is used to turn the retrieved cells into a glass-like state within seconds. This technique ensures that ice crystals do not form and therefore the frozen cells are not damaged during the thawing process.

"We have seen a substantial increase in cumulative live birth rate taking place over time," said Saket, "and this has happened in parallel with an increase in blastocyst transfer, particularly when used with frozen treatments. ■

Does Previous COVID-19 Infection Affect Success in IVF?

COVID-19 has caused concern in multiple therapeutic areas and bodily systems, including the reproductive system. Scientists aimed to explore how a previous infection with COVID-19 could potentially affect the chance of success in pregnancies occurring via *in vitro* fertilisation (IVF), and shared the findings in a press release for ESHRE 2021.

Scientists have discovered that receptors for severe acute respiratory syndrome coronavirus 2, namely the angiotensin-converting enzyme 2 receptor, are widely expressed in the ovaries. For this reason, there is anxiety in patients as to whether there is any link between previous COVID-19 infection and fertility treatment.

A recent study aimed to evaluate whether previous COVID-19 infection affected the chance of success in IVF. The study took place in a fertility clinic in Spain and included 46 patients. Patients awaiting IVF treatment had baseline hormones measured such as the anti-Müllerian hormone (AMH). AMH is an indicator of ovarian reserve; in recent times, AMH is measured in many clinics as a predictor of patient response to ovarian stimulation in IVF.

The results showed that there were no changes in AMH levels before and after the COVID-19 infections. From this, the researchers could infer that a previous infection of COVID-19 did not affect the chance of success in patients having IVF treatment. Although there was a small decrease in AMH in patients who were predicted to have a normal response to IVF, this was not enough to suggest that COVID-19 was behind this decline or that COVID-19 affected the chance of success.

Finally, the study concluded that COVID-19 does not impact fertility; this result is reassuring for patients who are worried about previous COVID-19 infections affecting the outcome of their IVF treatment. Despite these favourable results, it is important to consider the small sample size and question the robustness of the data. Nonetheless, other studies conducted so far in a similar field have supported the results in this study and, overall, the results are promising. ■

The results showed that there were no changes in AMH levels before and after the COVID-19 infections.

UK Study Presents Over-Retrieval of Oocytes in IVF

SURFACING information from a retrospective observational study in the UK has suggested that IVF clinics are retrieving too many oocytes, that a large number of these are not used, and have been discarded as a result. Findings focused on the number of eggs retrieved and IVF cycles; >1.625 million eggs in the UK were retrieved from 147,274 women between 2015–2018. Gulam Bahadur, North Middlesex University Hospital, London, UK, presented the research findings at ESHRE 2021, suggesting that the eye-opening discovery about UK clinics is likely reflected in other global practices.

Reasoning for the high egg retrieval lies in part with implementation of egg freezing, frozen embryo replacement cycles, and aggressive stimulation regimes, aimed to boost success rates in older women and those poor responders who produce fewer eggs. Researchers describe the unknown impacts of overstimulation practices on the health of patients, as well as upon their emotional health and financial wellbeing.

The report is extrapolated from study in all UK IVF clinics, relating to the non-donor fertility treatment carried out using 172,341 fresh oocyte retrieval cycles. An average of 11 eggs were collected per patient, 16% of cycles were associated with 16–49 oocytes retrieved per cycle, and 58 women had >50 eggs collected in a single egg retrieval procedure. Notably, the study discovered a large proportion (n=10,148) of cycles did not yield any oocytes. Of all IVF cycles, 53% were in the desired egg yield range of 6–15, one-quarter of all cycles resulted in 1–5 eggs, 14% produced 16–25 eggs, and only 2% yielded 26–49 oocytes. From all the eggs retrieved, a total of 931,265 embryos resulted to create a fertilisation rate of 57%; 22% of these were transferred into the uterus, and 24% were frozen. The remaining 43% unfertilised oocytes were likely to have been discarded in line with normal practice. The authors made a point to mention that of the 54% of embryos not transferred, a large majority will be discarded, after patients have paid for several years to maintain them in storage.

This study stimulates rethinking of current egg collection practices, highlighting the importance of recognising the ethical concerns at play, as well as procedural impracticalities, both of which should be carried forward by future study. This is summarised by Bahadur's statement: "Our observation suggests that the high oocyte number per retrieval procedure needs re-evaluation," including focus on "procedure-related complications, and on the fate of unused frozen oocytes and the costs associated with freezing them." Future practice is encouraged by this work to consider "financial and emotional cost," and to provide patients with "more information about the implications of freezing eggs and embryos." ■

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Artificial Intelligence Advancement Limits Need for Cell Biopsy in Embryo Testing

ARTIFICIAL intelligence (AI) and computer vision could be taking over *in vitro* fertilisation laboratory processes, especially in embryo testing for chromosomal content. Euploids are embryos with a normal complement of chromosomes, which have a better chance of implanting in the uterus leading to pregnancy, whilst aneuploid, abnormal embryos have barely any chance of implantation. A routine test in many fertility clinics is to test for aneuploidy, also known as PGT-A*, which requires samples of single or multiple cells acquired from the embryo via biopsy. As this method is invasive, a need for non-invasive methods has arisen.

A new study presented by Lorena Bori, IVIRMA, Valencia, Spain, at ESHRE 2021 on 28th June 2021, suggested that AI methodology using time-lapse imaging to observe cell activity could visually distinguish euploid embryos from aneuploid embryos, with no need for cell biopsy. Time-lapse technology over the past decade has revolutionised the visualisation of embryo growth by providing images throughout embryo development. Prior to this study, the time-lapse imaging technique has not been able to provide precise evaluation of an embryo's chromosomal content status. However, this study showed that a combination of computer vision with AI could provide an impartial and reliable prediction of embryo status before implantation.

The baseline of the study was that euploid embryos start their development to blastocysts slightly earlier than aneuploid embryos, and this could be identified via computer vision by the use of microscopic measurements of the cell's edges. This is an accurate method of calculating the number of cells and observing the blastomere cell cycle. The study, using computer vision-based measurements of cell edges and AI time-lapse videos, retrospectively compared 111 euploid and 120 aneuploid embryos. The results showed that despite the aneuploid embryos beginning their development to blastocysts slightly later than euploid embryos, their higher level of cell activity meant that aneuploid embryos achieve their growth to the blastocyst stage faster than the euploid embryos.

"Our results show for the first time," said the authors of the study, "that an AI-based system can precisely measure microscopic cell edges in the dividing embryo, which allowed us to distinguish between euploid and aneuploid embryos." ■

"...an AI-based system can precisely measure microscopic cell edges in the dividing embryo, which allowed us to distinguish between euploid and aneuploid embryos."

Patients Paying for IVF Add-On Treatments with Limited Evidence

ACCORDING to research findings presented by Sarah Lensen, Postdoctoral Research Fellow, Department of Obstetrics and Gynaecology, University of Melbourne, Australia, at ESHRE 2021, the majority of women undergoing *in vitro* fertilisation (IVF) are opting to pay for add-on therapies despite limited evidence supporting their effectiveness.

Data were collected through an online survey, which was advertised on Facebook, distributed to women undergoing IVF, and completed between June and July 2020. Gestational surrogates, patients who used a surrogate, and individuals who donated eggs or underwent elective egg freezing were excluded from the analysis. Participants were asked about their IVF and medical history, add-on use over the past 3.5 years, and what importance they placed on evidence concerning effectiveness and safety.

...the majority of women undergoing IVF are opting to pay for add-on therapies despite limited evidence supporting their effectiveness.

This retrospective study focused on a cohort of 1,590 Australian women. Overall, >72% had incurred additional costs from these unproven or experimental treatments. The most commonly used of these optional extras were acupuncture (45%), pre-implantation genetic testing for aneuploidy (28%), and Chinese herbal medicine (26%).

Only 18% of women reported having raised the issue of add-on options first. Over half (54%) of respondents stated that they had initially heard about them from their fertility specialist.

Additionally, 30% of women experienced moderate-to-severe regret about using add-ons compared with 34% who had no regret at all. The level of misgivings were higher among women who had failed to conceive or achieve a live birth.

Many women placed significant importance on scientific evidence corroborating the use of add-ons, in terms of either improving live birth rates (55%) or safety (73%). In reality, these adjunct treatments are generally not supported by evidence that they increase live birth rates. Indeed, some (e.g., immune therapies) are associated with low birth weight and other complications.

In summary, Lensen stated that the use of some adjunct therapies is likely to be "associated with a significant financial burden" and may "potentially pose risk to patient." For this reason, the Human Fertilisation and Embryology Authority (HEFA), which oversees the use of embryos and gametes in fertility treatment and research in the UK, has introduced a traffic light system to rate add-ons; to date, none have been awarded a green signal. Going forward, there is a need for such impartial evidence-based information in other countries. ■

No Increased Risk of Cancer Development in ART Children

RECENT evidence has emerged suggesting that children and young adults conceived through assisted reproductive technology (ART) show no increased risk of cancer development. These findings follow growing developments suggesting that ART procedures affect the normal genetic modifications that occur in the embryo before implantation. Results of this 18-year median follow-up study were presented at ESHRE 2021 on 28th June 2021 by Mandy Spaan, Amsterdam University Medical Center (UMC) and Netherlands Cancer Institute, Amsterdam, Netherlands. With dramatic increases in the number of children born through ART procedures in the last decade, studies such as this hold great importance for public health.

It was previously speculated that many aspects of the ART process, including the use of fertility drugs and embryo freezing and thawing, could have a potentially harmful impact on the functioning of embryos through epigenetic changes. The median follow-up study carried out included 89,249 children born by subfertile women between 1980 and 2012, 51,417 of which were conceived through different means of ART. Analysis demonstrated that over the 18-year study, 358 cancers were diagnosed in children: 157 in the ART group and 201 in the non-ART group. This evidence reveals that there was no increased risk of malignant cancer development in ART-conceived children compared to those conceived naturally.

When delving deeper into the various types of ART, however, it was observed that children conceived by intracytoplasmic sperm injection (ICSI) had a higher risk of developing cancer. Authors have revealed that this may have been down to chance and was likely driven by an increased risk of melanoma development, as seen in four cases. There was also no increased risk of cancer development in those conceived through frozen embryo transfer (FET) compared to fresh embryo transfer. Spaan described these results as "an important contribution to current knowledge about health risks in ART offspring," which will provide both parents and clinicians with evidence regarding potential cancer risks in ART-conceived offspring. ■

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Accurately Predicting Ovarian Failure in Girls with Cancer

"To summarise, Howie highlighted that the Edinburgh criteria "are a robust tool for selecting those at high risk at the time of diagnosis who should be offered OTC." "

CANCER treatments, such as chemotherapy and radiotherapy, can damage the ovaries, causing primary ovarian insufficiency (POI). Although ovarian tissue cryopreservation (OTC) can be offered to safeguard future fertility, it is invasive, has risks, and evidence suggests that most girls do not develop POI (the prevalence after childhood cancer is estimated at 10%). Consequently, a selection tool has been developed to accurately predict ovarian damage in girls under 18 years of age. Ruth Howie, University of Edinburgh and NHS Lothian, Edinburgh, UK, presented the research findings at the 37th ESHRE virtual Annual Meeting 2021.

Results were obtained from a long-term follow-up study of 423 girls and young women. Nearly 25% (n=9) of the 37 individuals classified as high-risk by the Edinburgh selection criteria were diagnosed with POI. Conversely, only 3% of the 386 patients categorised as low-risk developed premature ovarian failure. The Edinburgh selection criteria for OTC, based on knowledge of relevant scientific literature and clinician experience of POI, were developed by a multidisciplinary group of experts and have been in use since 1996.

Data were based on all females (<18 years) diagnosed with cancer in Southeast Scotland between 1st January 1996 and 30th April 2020. Reproductive function was assessed by evidence of puberty, hormonal measurements, the presence of menstruation, pregnancy, or POI diagnosis at most recent follow-up to October 2020. In the present study, the average time for developing POI was 5.8 years.

Of the 639 individuals diagnosed, approximately 34% (n=216) were excluded from the analysis, including those on hormonal contraception, girls aged under 12 years, and individuals who died before the age of 12 years. Of the 423 remaining patients, more than a third (n=143) had unknown reproductive outcomes.

Therefore, a subgroup analysis of 280 patients was performed in addition to the primary analysis. Twenty-nine of the 280 individuals were identified as high-risk of POI and offered OTC, with 31% (n=9) subsequently developing the condition. In contrast, only 4% (n=11) of the 251 low-risk patients developed the condition.

To summarise, Howie highlighted that the Edinburgh criteria "are a robust tool for selecting those at high risk at the time of diagnosis who should be offered OTC." Even so, it is important to continuously review the criteria via long-term follow-up of the reproductive outcomes of those assessed. ■

The Evolution of the World Health Organization (WHO) Manual on Semen Analysis

Janet Nzisa
Editorial Assistant

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ON DAY 4 of the European Society of Human Reproduction and Embryology (ESHRE) Congress 2021, participants worldwide virtually joined a session on semen analysis (SA) entitled "Evolution of the WHO Manual: Where Are We Now?" chaired by Christopher De Jonge, Director of the Andrology Programme at the University of Minnesota Medical Center, USA, and Ellen Goossens, Professor at Vrije Universiteit Brussel, Belgium.

EVOLUTION OF THE WHO MANUAL

Sandro Esteves, Medical Director at ANDROFERT Clinic, Campinas, Brazil, began the session by defining male infertility as the disease that affects the male reproductive system that is primarily caused by genetic and congenital conditions, anatomical, endocrine, functional or immunological abnormalities, chronic illness, certain lifestyle factors, and sexual-related conditions. These factors could be associated with semen deficiencies and may not be detected during SA. Currently, routine SA is the only test carried out to determine an individual's fertility potential. However, male fertility evaluation solely based on the results of routine SA is inadequate in the identification of underlying conditions associated with infertility. A recently published study by Esteves explicated that male fertility primarily detected through routine SA does not provide males with an optimal fertility pathway. Firstly, the reference intervals in routine SA do not accurately distinguish fertility from subfertility. Secondly, the results procured from an individual, unless at extremely lower limits, have a restricted prognostic value in both natural and assisted conception. Furthermore, routine SA does not detect the defects in sperm DNA that may adversely influence embryo implantation, development, and overall offspring health.

The European Association of Urology (EAU) 2021 guidelines state that male and female partners should be simultaneously assessed during the initial fertility evaluation. The evaluation in males should not be limited to SA only but should also include a medical and reproductive history,

physical examination, sperm function test, and endocrine evaluation. Additional testing such as genetic evaluation, transrectal ultrasound, retrograde ejaculation assessment, testicular biopsy, and vasography may be required depending on the clinical characteristics and semen parameters of the individual. A study by Schlegel et al. states that the objectives of a male fertility work-up are to identify the following types of conditions:

- a potentially correctable condition;
- irreversible conditions suitable for assisted reproductive technology (ART) using the individual's sperm;
- conditions in which donor insemination or adoptions are the main options;
- any genetic abnormalities that may affect the health of a patient or offspring, especially if ART is used.

Esteves emphasised that SA is fundamental in the evaluation of the male fertility component and provides valuable information about spermatogenesis, epididymides, and the accessory sexual glands. Furthermore, SA could help in the diagnosis of defects in the male reproductive organs, follow-up in male infertility treatment, and even the selection of suitable ART treatment modality. SA is a highly complex laboratory test and therefore should be carried out exclusively in an andrology laboratory by experienced technicians. Additionally, internal and external quality controls, validation of test systems, and quality assurance are aspects to be considered when choosing a laboratory to carry out SA.

Since the 1980s, the Department of Reproductive Health and Research of the World Health Organization (WHO) has been publishing guidelines on how the assessment of human semen should be carried out in the laboratory. These guidelines are updated periodically and incorporate the latest evidence derived from research. The manual for the examination and processing of human semen includes a detailed protocol, reference standard values, and other relevant information. The current fifth edition of the manual was published in 2010 and can be found on the WHO website. This manual contains guidelines for the examination and processing of human semen utilised by technicians in clinical and research laboratories and consists of three main sections: SA, sperm preparation, and quality assurance.

"... SA is fundamental in the evaluation of the male fertility component and provides valuable information... "

Esteves explained that the authors of the fifth edition WHO guidelines on human sperm assessments relied on clinical chemistry principles to generate 95% intervals for sperm volume, count, motility, vitality, and morphology from recent fathers (≤12 months). A total of 1,953 individual male sperm specimens were used to generate these data. These values have been used in clinical practice and research; however, when compared to previous editions of the WHO manuals, the 2010 standards were marked lower. The previous editions relied on the clinical experience of the authors who had studied populations of males considered healthy and fertile without the presence of a pregnancy. Therefore, the quality of data used in previous WHO editions to generate reference values could not be contended and therefore was not reliable. Nevertheless, the reference values of the fifth edition have raised concerns about its clinical utility and general practicality and have been the subject of numerous debates. Criticisms include the limited geographical area of the participants, the methods used in SA, and the potential impact of the new reference ranges. Esteves further explained that the data used to generate the 2010 WHO manual references values were most exclusively collected from participants who lived in countries located in the northern hemisphere. Furthermore, the data used to produce these values were from a group of investigators who worked collaboratively, therefore it is not clear to what extent the information represents the global semen characteristics of a male who is considered to be fertile. Nearly 40% of the male infertility population previously classified as having abnormal levels of SA values based on the 1999 WHO manual reference values were reclassified as having SA references values within the normal range in the 2010 WHO manual guidelines. These results further highlight that male infertility testing primarily based on routine SA does not provide a guide towards an optimal fertility pathway.

SPERM DNA FRAGMENTATION TESTING

Esteves presented a meta-analysis study that his team carried out on the effect of cigarette smoking on semen quality, to analyse the impact of 2010 WHO guidelines of SA. The results showed that smoking negatively impacted sperm morphology when the reference values of the 1999 or earlier WHO manuals were used; however, this was not the case when the 2010 WHO manual methodology and reference values were used. As stated by Esteves, approximately 30% of males with unexplained male infertility present with sperm deficiencies that can only be recognised through sperm functionality examinations such as assessment of DNA integrity and oxidative stress tests. In another study by Esteves et al., 70% of patients had high sperm DNA fragmentations despite having sperm parameters within the normal range according to the WHO manual guidelines. Therefore, it is clear that integration of other tests alongside the routine SA tests are required and may add valuable information in fertility counselling, diagnosis, and treatment planning. The sperm DNA fragmentation tests could assist in identifying underlying conditions that affect male fertility. Another study by Esteves showed that sperm DNA fragmentation could be treated by avoiding factors that contribute to oxidative stress such as smoking, obesity, and environmental factors.

Oral antioxidant therapies and supplements such as vitamin C and E, folic acid, selenium, zinc, and co-enzyme Q10, among others, could be beneficial in improving male fertility. Another approach involves the treatment of underlying conditions that cause male infertility, e.g., genital tract infections, leukocytospermia, varicocele repair, diabetes, thyroid disorders, and hyperprolactinaemia. Follicle stimulating hormone therapy is advantageous in the regulation and production of sperm in males affected by infertility. Furthermore, intracytoplasmic sperm injection and ART are effective interventions for men affected by infertility. Esteves emphasised that fertility specialists, reproductive urologists, and clinical andrologists should perform male fertility evaluations by carrying out a detailed history examination, physical examination, routine SA, and other tests as needed.

WHO MANUAL UPDATE 2021

The upcoming sixth edition of the WHO laboratory manual for the examination and processing of human semen includes essential updates. Esteves stated the draft was made available in March 2021 for public review, and he compared it to the previous editions. Chapters

on tests that are no longer essential like sperm cervical penetration, assays, and hamster egg penetration have been removed. Alternatively, the advanced tests section has been extensively improved with comprehensive information covering sperm DNA fragmentation tests, markers for genital tract inflammation, sequence for ejaculation, semen biochemistry, and sperm aneuploidy assessments, among other updates. Research procedures such as oxidative stress test, acrosome reaction test, and sperm chromatin structure and stability procedures have also been extensively covered. Furthermore, Esteves highlighted that the sections related to sperm processing, cryopreservation of spermatozoa, and quality control and assurance are now more detailed. Each of these updated sections contains a variety of protocols for processing both ejaculated and surgically extracted sperm and includes details on sperm vitrification techniques.

Additionally, novel SA data have been included in the draft sixth edition of the WHO manual. The authors acquired new data from 1,789 males from five studies carried out in China, Egypt, Greece, Italy, and Iran. These new figures were combined with the reference value data used in the fifth edition, with a total of 3,589 specimens, providing new reference intervals in the upcoming WHO manual. Despite the number of specimens being twice as high compared to the previous WHO manual, the number of studies is still inadequate because certain geographical regions have not been represented. No study from South America has been included and only one research study has been carried out in the entire African continent. The number of participants is still insufficient, despite the significant increase compared to previous manuals, and the diversity in participants is also limited.

In conclusion, Esteves explained that the terminology in the upcoming sixth edition of the WHO manual used to describe low sperm concentration, motility, and morphology will also have to be qualified depending on the new reference values indicating conditions such as oligo-astheno-teratozoospermia. He suggested the clinicians who interpret SA should utilise the methods used within his institution and compare the patients' data with the lower (5%), median (50%), and upper (95%) reference values provided by WHO manuals. A physical examination and clinical history should also be carried out to determine if additional tests, to further guide clinical management, and counselling are required. These thorough tests may reveal underlying conditions, which could be treated and potentially improve the health of patients and their offspring.

The Future of Endometrial Preparation: Challenges and Opportunities

This symposium took place on the 28th June 2021 as part of the 37th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), which was held virtually between 26th June and 1st July 2021

Speakers: Annalisa Racca,[1] Dominique de Ziegler[2]

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Meeting Summary

The landscape of assisted reproductive technology (ART) is shifting, with an increasing move toward frozen embryo transfers (FET). Multiple studies have confirmed the pivotal role of progesterone in the establishment and maintenance of pregnancy. However, challenges arise when applying progesterone supplementation protocols developed for endometrial preparation in fresh transfers to the new 'freeze-all' era. In particular, clinical questions surround the efficacy of vaginal progesterone in FETs, with one-third of patients failing to achieve sufficient levels of this key hormone using standard micronised vaginal progesterone (MVP) supplementation. In this symposium, leading experts in reproductive medicine considered alternative routes of progesterone administration and discussed the best strategy to rescue a cycle in cases of inadequate levels, with the overall aim of optimising reproductive outcomes after FET. In the new freeze-all era, self-administered subcutaneous (SC) progesterone injections offer a valid and efficient alternative to intramuscular (IM) and vaginal progesterone preparations for luteal-phase support in FET, which obviates the need for ongoing progesterone level monitoring. Emerging evidence indicates that a single SC progesterone injection (25 mg/day) can effectively rescue the cycle at any point during the luteal phase, and twice-daily injection is equivalent to IM progesterone (50 mg/day) in priming for FETs.

New Challenges of the Freeze-All Era

Annalisa Racca

The last decade has seen a sharp increase in the number of freeze-all cycles and subsequent FETs. Annalisa Racca outlined the challenges and opportunities presented by the freeze-all era, focusing on the key role of progesterone assessment and supplementation to maximise FET cycle success. 'One size fits all' no longer applies in the freeze-all era, explained Racca; instead, each female needs to be managed individually in order to optimise reproductive outcomes.

Improvements in laboratory techniques over recent years have led to a considerable rise in the number of supernumerary embryos being frozen after a fresh cycle. There has also been a sharp upsurge in the number of freeze-all cycles carried out for both elective and non-elective reasons (e.g., the COVID-19 pandemic). The net result is that ever-increasing numbers of FETs are being performed. Figures from a UK registry revealed a 707% increase in the number of freeze-all cycles carried out over the 5-year period to 2018. A total of 7,031 freeze-all cycles were carried out in 2018, compared to just 871 in 2013.[1]

The key advantage of the freeze-all era is that it allows reproductive medicine to be viewed not just in the context of a single child, but as an overall family project. However, every big change that happens in medicine also brings significant challenges, noted Racca. With the freeze-all era, these challenges lie in the need to adapt and develop better strategies in order to achieve the same or higher pregnancy and live birth outcomes with FET as with fresh embryo transfers. FET can be performed in a natural cycle, in a modified natural cycle where ovulation is induced with human chorionic gonadotropin (hCG), or in an artificial cycle, the so-called hormone replacement therapy (HRT) cycle. No one approach has been demonstrated to be superior to the other, as all have specific pros and cons.

The HRT cycle using an artificially prepared endometrium is the most widely used worldwide, and benefits from minimal cycle monitoring and easy scheduling. This approach can be applied equally to every single female at any age or stage in their reproductive journey, confirmed Racca. Disadvantages include cost, inconvenience, and the potential thrombotic side effects of estradiol (E2). The two main players in HRT cycles are the oestrogens and the progesterones. Studies looking at priming of the endometrium with E2 have shown no impact of length, dose, or serum level on cycle outcomes including clinical pregnancy rate and miscarriage rate.[2-4] So, while oestrogens are obviously important, they are not as crucial in dictating cycle outcomes as progesterone, concluded Racca.

Progesterone plays several critical roles in human reproduction, facilitating transformation of the endometrium into a receptive environment and performing the dual functions of immunomodulation and myometrial quiescence, which are vital in reducing the risk of preterm deliveries. However, the optimal way to administer and measure progesterone in FETs remains a key question, said Racca. It is important to be aware that different administration routes for progesterone (vaginally, IM, or SC) will result in differing pharmacokinetic profiles. Studies have confirmed that serum concentrations of progesterone are higher with SC or IM administration, while endometrial concentrations are higher with vaginal dosing.[5] Irrespective of the route of administration, significant variability in progesterone levels is also evident throughout the course of a day during both the luteal phase and the late follicular phases.[6,7]

When is best to assess progesterone during the luteal phase is another critical question in ART. In a study where progesterone assessment was performed the day before FET, a significantly lower live birth rate (47.5%) was observed in patients with a progesterone value <10.64 ng/mL compared to those with a progesterone level above this threshold (62.3%; p=0.017).[8] Another study that measured progesterone on the day of FET found a lower pregnancy rate in patients with progesterone values <9.2 ng/mL (the pregnancy rate was 32.7% below this cut-off and 52.8% above it).[9] A third study where progesterone was assessed 11–12 days after FET confirmed that progesterone levels <35 nmol/L, corresponding to approximately 10 ng/mL, were associated with a lower pregnancy rate (38% versus 51%, respectively).[10]

These data clearly demonstrate that, regardless of the timing of progesterone assessment, outcomes from the cycle are consistently improved with higher progesterone concentrations, stressed Racca. However, available evidence indicates that a substantial proportion of patients undergoing FET fail to achieve adequate levels of this essential hormone. One-third of those receiving MVP showed inadequate levels of progesterone in a recent 2020 study. Progesterone concentrations were found to be suboptimal (<8.8 ng/mL) in around one-third of cases across all the different cycles performed, including pre-implantation genetic testing for aneuploidy (PGT-A) or non-PGT-A with own oocytes and oocyte donation.[11]

Given the evidence that progesterone is a key driver of cycle outcomes, several studies have explored the important question of how and when to use progesterone supplementation to rescue a FET cycle. In a retrospective study of 227 FET cycles, inadequate serum progesterone levels <10 ng/mL were identified in 37% of cycles on embryo transfer day, after participants received 600 mg/day vaginal supplementation.[12] In these people with suboptimal progesterone, MVP dose was increased to 1,200 mg/day and blood levels retested 2 days later. However, progesterone concentrations remained below the key 10 ng/mL threshold in 31% of women on re-evaluation, despite doubling of the dose of vaginal supplementation.[12] Racca suggested that this could be due to malabsorption of vaginal progesterone, pharmacokinetics, saturation of the receptors, or the influence of the microbiota. It is not known why, she conceded, but it can be seen from this study that doubling the dose of vaginal progesterone is "not the ideal solution."

A further retrospective study investigated combined supplementation with SC and vaginal progesterone from Day 1 of the luteal phase in 320 FET cycles conducted in 213 females.[13] By using combined vaginal (800 mg/day) and SC (25 mg/day) doses, 95% achieved progesterone levels >10.5 ng/mL, with a minimum value of 7.2 ng/mL. Analysis of outcomes by progesterone quartiles revealed higher ongoing pregnancy rates (35.6% versus 26.3%) and lower miscarriage rates (12.3% versus 27.6%) in the upper two quartiles of serum progesterone (>21.95 ng/mL) compared to the lower quartiles.[13]

Racca went on to describe findings from a prospective observational study carried out by her own group, which investigated rescue of the FET protocol with daily SC progesterone injections in 574 HRT cycles (453 patients).[14] In this study, serum progesterone was assessed the day before FET and, if found to be below the key threshold of 10.6 ng/mL, was supplemented from the day of embryo transfer with SC progesterone. Overall, 38% of women were found to have inadequate progesterone levels with standard vaginal supplementation, but >98% were able to reach levels >10.6 ng/mL with SC progesterone injections. Females who received daily SC progesterone injections started the day prior to FET achieved similar reproductive outcomes compared to those with initial adequate progesterone levels, with 'excellent' ongoing pregnancy and live birth rates (Figure 1).[14] These findings show that there is a 'window of opportunity' where we can rescue the protocol and safeguard reproductive outcomes, even on the day of the FET itself, noted Racca.

Other key issues to consider in the freeze-all era include whether single measurements of progesterone are sufficient or if repeated testing is more meaningful, and how late in the luteal phase a cycle can still be rescued. To answer these questions, Racca's team conducted a further study in which progesterone assessments were performed both the day before FET and on the day of the hCG test itself.[15] Results revealed that, even in the population of women with normal progesterone on the day prior to FET, 30% had dropped to inadequate progesterone levels by the day of the hCG. This fall in progesterone levels was evident regardless of the specific treatment they were undergoing (i.e., RECEP, PGT-A, or CT Propios). In this study, rescue of the protocol with SC progesterone even as late as the day of hCG in women with inadequate progesterone levels was still able to save the cycle.[15] Racca described these findings as 'striking', with miscarriage rates consistently above 60% in the women with progesterone levels <10.6 ng/mL who did not receive supplementation, compared to low single-digit miscarriage rates in those whose cycles were rescued with SC progesterone.[15]

Overall, this collective clinical evidence confirms that luteal progesterone is a very strong predictor of cycle outcomes in FET HRT. Yet one-third of females receiving standard MVP supplementation still show inadequate levels of progesterone in any of their luteal measurements. Individualisation of progesterone supplementation in FET HRT and application of rescue protocols, such as SC progesterone injections, are therefore vitally important, concluded Racca, and can be implemented at any point during the luteal phase

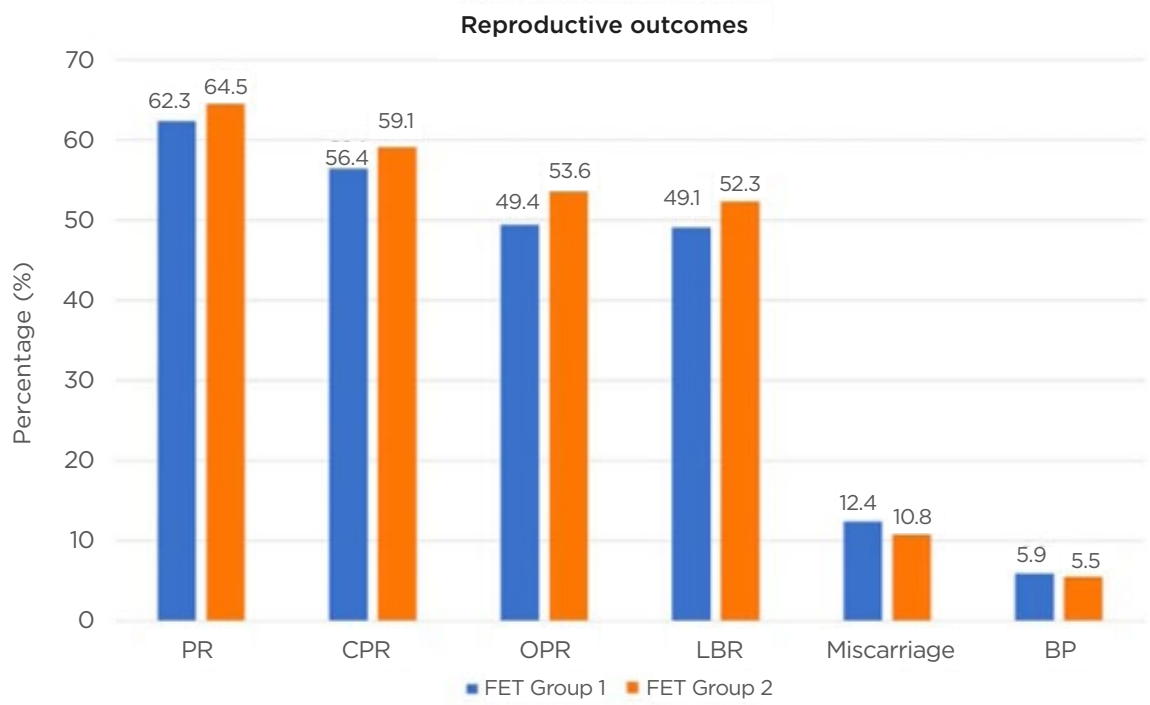

Figure 1: Subcutaneous progesterone injections can rescue the frozen embryo transfers protocol in cases of low progesterone.[14]

BP: biochemical pregnancy; CPR: clinical pregnancy rate; FET: frozen embryo transfer; LBR: live-birth rate; OPR: ongoing pregnancy rates; PR: pregnancy rate; SC: subcutaneous.

Tomorrow's Perspectives for Endometrial Preparation

Dominique de Ziegler

In this talk, Dominique de Zeigler outlined key problems that have been encountered in the increased shift towards frozen from fresh embryo transfers and shared potential solutions for adapting existing FET regimens to optimise endometrial preparation moving forward, based on available clinical evidence. None of the progesterone preparations currently available and in clinical use have ever been formally tested in FET, stressed de Zeigler. Ovarian progesterone production also shows a progressive increase during the early weeks of pregnancy, which must be compensated for during FET.[16]

There is a clear contrast in the underlying hormone profiles encountered in fresh versus frozen ART. In fresh embryo transfers, the key issue is decreased production of progesterone during the luteal phase arising from the impact of treatment on the pituitary gland. However, as soon as the patient becomes pregnant, normal progesterone production by the corpus luteum resumes under activation by hCG. The progesterone support provided usually continues until the luteo-placental shift, although de Zeigler emphasised that this is not really necessary as, in fresh cycles, progesterone can actually be stopped on the day of the positive pregnancy test itself without detrimental consequences.

However, in FET, the situation is completely different because there is no endogenous hormone production, explained de Zeigler, which must be supplied in the form of E2 and progesterone supplementation regimens. Progesterone supplementation is required to cover not only the luteal phase, but also the increased production that occurs in the early weeks of the pregnancy. The amount of progesterone delivered therefore has to be increased, stressed de Zeigler, and while 25 mg/day is sufficient in fresh cycles, a higher dose, such as 50 mg/day, may be required to achieve

optimal outcomes in FET. Failure to achieve adequate levels of progesterone has detrimental consequences, he added.

Although vaginal progesterone has been used for many years and is approved for fresh embryo transfers, failure to achieve adequate blood levels when used in FET is a common clinical problem. This shortcoming in vaginal progesterone regimens may require rescue options to be initiated if progesterone levels in a FET cycle fall too low. As illustrated by Racca, evidence shows that lower quartiles of progesterone on the day of embryo transfer, notably serum levels <9.2 ng/mL, are associated with worse reproductive outcomes, including a diminished ongoing pregnancy rate and a higher incidence of miscarriage.[9] This is a clear limitation of vaginal progesterone designed for fresh transfer when used in the FET setting, remarked de Zeigler, and the core issue which must be solved in the modern freeze-all era.

Evidence from a recent study has shown the efficacy of SC progesterone in providing luteal-phase rescue in FET HRT cycles.[17] In this study, progesterone was measured the day prior to embryo transfer and, if <8.75 ng/mL, was rescued with exogeneous progesterone provided in the form of daily SC injections at a dose of 25 mg. Following addition of SC progesterone, serum progesterone rose to 33.4 ng/mL on the day of embryo transfer and equivalent clinical pregnancy rates were achieved in the rescue group to the control arm: 55.0% versus 56.7%, respectively.[17] Data from another study have also demonstrated the efficacy of SC progesterone given at double dose of two daily 25 mg injections in priming for FET. In this retrospective trial involving 214 women undergoing FET, priming with either SC progesterone (25 mg twice daily [BID]) or 90 mg vaginal gel (also given BID) resulted in equivalent reproductive outcomes. The live birth/ongoing pregnancy rate per embryo transfer was 39.3% with SC progesterone 50 mg BID versus 35.5% with vaginal supplementation.[18]

The only viable administration routes for progesterone in ART are parental and vaginal; oral and transdermic delivery are contraindicated due to poor bioavailability and permeability, respectively. IM injections were developed first for ART protocols but must be carried out by a nurse and are recognised to be painful by patients. The vaginal route was therefore introduced as an alternative. This results in a direct transfer of progesterone, leading to high local concentrations in the uterus, yet appears to underperform in FET, with around one-third of patients failing to reach sufficient levels of progesterone in the serum.

These problems encountered with vaginal progesterone have led the majority of groups in the USA to revert back to using exclusively IM progesterone for FET, or a combination of IM/vaginal regimens. However, de Ziegler explained that in the modern era of ART there is now access to a new injectable option for progesterone: an aqueous solution that has been developed specifically for SC delivery (Prolutex®; IBSA Institut Biochimique SA, Lugano, Switzerland). In this formulation, progesterone has been encapsulated in cyclodextrin, a polar substance long-used for enhancing the solubility of drugs, to overcome its inherent hydrophobicity. Once injected, the cyclodextrin envelope (a starch residue) is readily digested and progesterone released.

Pharmacokinetic analyses have compared SC progesterone 25 mg administered BID against both vaginal progesterone (90 mg) and IM progesterone (50 mg) given once daily (data on file). Currently, the SC progesterone preparation is approved at doses of 25 mg daily for fresh embryo transfers, but in FET "we need more," reiterated de Ziegler. In this pharmacokinetic study, BID administration of SC progesterone achieved a higher rate of absorption compared to controls and delivered similar trough levels to IM injection (data on file).

Looking at clinical outcomes, SC progesterone (25 mg BID) was compared to IM progesterone (50 mg/day), the standard dose used at most ART centres, in a recent retrospective study involving >500 patients.[19] Both formulations of progesterone were found to be equivalent in terms of pregnancy rate, live birth rate, and miscarriage rate.[19] de Ziegler described this as a "major finding," validating a new option for effective progesterone supplementation that avoids the painful IM injection and replaces it with a simple, self-administered SC injection, while simultaneously circumventing the well-recognised shortcomings of vaginal dosing.

A further prospective study, presented at the European Society of Reproduction and Embryology (ESHRE) Annual Meeting 2021, compared luteal support with IM progesterone 50 mg (n=92) to SC progesterone 25 mg BID (n=133) and also explored the association between progesterone blood levels achieved with each dosing route and outcome.[20] Contrary to the clinical experience with vaginal dosing, there was no difference in ongoing pregnancy rate between the different progesterone level groups when ranked by quartiles in patients treated with SC progesterone.[20] In all cases, the serum levels of progesterone reached with twice-daily SC injection were sufficient, explained de Ziegler, thereby negating the need for ongoing progesterone level monitoring in FET cycles. The same benefit was not seen for IM dosing, where progesterone levels on the day of transfer were still found to have a significant impact on pregnancy rate (p=0.02).[20] This study also showed similar reproductive outcomes were achieved by patients treated with either daily IM or twice-daily SC progesterone injections.[20] Clinical pregnancy rates were 64.7% versus 62.6% (p=0.757), miscarriage rates were 24.4% versus 17.5% (p=0.329), and ongoing pregnancy rates were 48.9% versus 51.6% (p=0.683), respectively.[20]

Although vaginal progesterone has been validated in fresh cycles as we move toward the freeze-all era and most of us now perform approximately 60% FETs, remarked de Ziegler, it must be acknowledged that vaginal progesterone has reached its limits. This poses an intellectual problem: why do low circulating blood levels of progesterone matter when we know high tissue concentrations are achieved with vaginal supplementation? de Ziegler suggested that the answer may lie in the pelvic and non-pelvic effects of progesterone. Progesterone not only acts on the uterus, but also works outside of the pelvis, where it exerts immunotolerance effects on non-pelvic organs, including the bone marrow and lymphocytes, adrenals, and the liver. This immunomodulation is a non-pelvic effect dependent on blood levels of progesterone and could therefore be the 'Achilles heel' of vaginal progesterone, de Ziegler proposed.

SC self-injected progesterone administered at a dose of 25 mg BID should therefore be considered the 'true replacement' for IM progesterone in FET cycles, de Ziegler concluded. SC injections provide a valid and efficient alternative to painful IM injections, which benefit from patient-friendly administration and have demonstrated comparable efficacy in priming for FETs. If using vaginal supplementation, an alternative solution is to measure progesterone and, if levels drop too low, rescue the cycle with SC progesterone, or use a combination regimen of vaginal plus one injection of SC progesterone daily in all patients, suggested de Zeigler, thereby eliminating the need to monitor progesterone at all.

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Abstract Reviews

Sharing insights and updates from a selection of abstracts presented by leading experts in the field of reproductive health at the European Society of Human Reproduction and Embryology (ESHRE) 2021

Reproductive Performance of Females With and Without Intrauterine Adhesions Diagnosis

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Keywords: Asherman syndrome, conception, dilatation and curettage (D&C), hyaluronic acid, intrauterine adhesions (IUAs), miscarriage, pregnancy, reproductive outcome.

Citation: EMJ Repro Health. 2021;7[1]:34-36. Abstract Review No. AR1.

BACKGROUND AND AIMS

Intrauterine adhesions (IUAs) are considered one of the main reproductive system diseases affecting females worldwide, characterised by endometrial fibrosis with partial to complete obliteration of the uterine cavity and/or cervical canal.[1-4] The Prevention of Adhesions Post Abortion (PAPA) study showed that application of auto-crosslinked hyaluronic acid (ACP) gel, an absorbable barrier in those undergoing recurrent dilatation and curettage (D&C) for miscarriage, resulted in a lower rate of IUAs, 13% versus 31% (relative risk: 0.43; 95% confidence interval [CI]: 0.22–0.83), lower mean adhesion score and significant less moderate to severe IUAs.[5] After a follow-up, the question remains if the long-term reproductive outcomes following recurrent D&C for miscarriage in individuals with identified and treated IUAs are comparable to individuals without IUAs.[6]

METHODS AND MATERIALS

This was a follow-up of the PAPA study, a multicentre randomised controlled trial evaluating the application of ACP gel in females undergoing recurrent D&C for miscarriage.[5] In the trial, 152 females who had undergone a first-trimester miscarriage with at least one previous D&C were randomised for D&C alone or D&C with immediate intrauterine application of ACP gel. All included females who had received a diagnostic hysteroscopy 8–12 weeks after randomisation to evaluate the uterine cavity and for adhesiolysis if IUAs were present. Participants were approached at least 30 months after randomisation to evaluate reproductive performance, obstetric, and neonatal outcomes. The main outcome was ongoing pregnancy. Outcomes of subsequent pregnancies, time to conception, and time to live birth were also recorded.

RESULTS

Reproductive outcomes of individuals with and without an IUA diagnosis after a mean follow-up of 46 months are presented in Table 1. In those pursuing a pregnancy, 14/24 (58%) ongoing pregnancies were recorded in individuals with identified and treated IUAs versus 80/89 (90%) ongoing pregnancies in people without IUAs diagnosis (odds ratio [OR]: 0.18; 95% CI: 0.06–0.50, p<0.001).[6] Documented live birth was also lower in those diagnosed with IUAs; 13/24 (54%) with versus 75/89 (84%) without IUAs diagnosis (OR: 0.22; 95% CI: 0.08–0.59; p=0.004). The median time to conception was 7 months in females with identified and treated IUAs versus 5 months in females without IUAs diagnosis (hazard ratio: 0.84; 95% CI: 0.54–1.33) and time to conception leading to a live birth 15 months versus 5 months (hazard ratio: 0.54; 95% CI: 0.30–0.97). In females with identified and treated IUAs, premature deliveries were recorded in 3/16 (19%) versus 4/88 (5%) in females without IUAs; p=0.01. Complications were recorded in respectively 12/16 (75%) versus 26/88 (30%); p=0.001.[6] No differences were recorded in mean birth weight between the groups.

CONCLUSION

Reproductive outcomes in females with identified and treated IUAs following recurrent D&C for miscarriage are decreased compared to females without IUAs diagnosis. As IUAs have an impact on reproductive performance, even after hysteroscopic adhesiolysis, primary prevention is essential.

Table 1: Reproductive outcomes of females with and without intrauterine adhesions diagnosis.

	Females diagnosed with IUAs (n=26)	Females without IUAs diagnosis (n=105)	P
Conceived	24/26 (92.3)	87/105 (82.9)	0.36
Ectopic pregnancy	2/26 (7.7)	0/105 (0.0)	0.04
Termination of pregnancy	0/26 (0.0)	4/105 (3.8)	0.58
Miscarriage	11/26 (42.3)	27/105 (25.7)	0.15
Ongoing pregnancy	14/26 (53.8)	79/105 (75.2)	0.05
Live birth	13/26 (50.0)	75/105 (71.4)	0.04
Females pursing a pregnancy	(n=24)	(n=89)	
Conceived	24/24 (100.0)	88/89 (98.9)	1.000
Ectopic pregnancy	2/24 (8.3)	0/89 (0.0)	0.040
Termination of pregnancy	0/24 (0.0)	4/89 (4.5)	0.580
Miscarriage	11/24 (45.8)	27/89 (30.3)	0.220
Ongoing pregnancy	13/24 (54.2)	80/89 (89.9)	<0.001
Live birth	13/24 (54.2)	75/89 (84.3)	0.004

IUAs: intrauterine adhesion

Expectative and medical management should therefore be considered as serious alternatives for D&C in females with a miscarriage. In case D&C is necessary, application of ACP gel should be considered.

LIMITATIONS AND REASONS FOR CAUTION

In the original PAPA study, randomisation was applied for ACP gel application. Comparing females with and without IUAs diagnosis is not in line with the randomisation and therefore confounding of the results cannot be excluded. IUAs, if visible during routine hysteroscopy after randomisation were removed as part of the study protocol. ■

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Signatures from the Father: Epigenetic Implications of Paternal Lifestyle, Exposure to Pollutants, and Advanced Paternal Age

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Keywords: Father-child effects, epigenetics, paternal origins of health and disease (POHaD).

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BACKGROUND AND AIMS

A number of findings in animals and some in humans support the notion that plasticity of the (epi)genome allows adaptation to environmental changes, resulting in altered genetic programming and phenotypic changes in the offspring. In this regard, a new paradigm was introduced: the paternal origins of health and disease (POHaD).[1,2] This theory explains how the paternal germ line may respond to environmental traits and how this may affect offspring health.

MATERIALS AND METHODS

There are significant associations between urinary levels of metabolites originating from exposures to indoor pollutants, such as flame-retardants or mixtures of endocrine disruptors (EDCs), and alterations in epigenetic patterns at differentially methylated regions of several imprinted genes in sperm of young males.[3] In brief, the data suggested that males exposed to mixtures of EDCs acquire DNA methyl groups at sites that are normally only methylated in the oocyte, but not in sperm cells. This 'feminisation' of epigenetic patterns in sperm (due to exposure to EDCs) needs further exploration.

Next, the author found that sperm cells of males who are obese or with an unhealthy lifestyle (such as frequent consumption of fatty foods) have a different epigenetic signature at the level of imprinting control regions, compared to sperm of males with a healthier weight or males with a healthy diet (including whole grain bread, vegetables, etc.).[4] This is consistent with findings in animal experiments. For instance, diet-induced epigenetic changes in rodent sperm have been linked to fertility issues in the exposed fathers or metabolic disturbances in the next generations.[5-7]

RESULTS

Although epidemiologic studies indicate that epigenetic signatures from fathers (who are obese) can be inherited,[8,9] it is currently unclear if these paternally induced effects on gene-regulation are to the extent that children will develop a chronic disorder in later life. The author's first studies show that the affected genes (by environmental exposures) are important in regulation of early growth. Hence, the author further explored potential effects of the father on embryo growth, through a prospective *in vitro* fertilisation study cohort.

While studies on influences from extrinsic exposures are still ongoing, the author's first data show that an intrinsic exposure factor, such as ageing, may affect the embryo epigenome and its growth. In the *in vitro* fertilisation cohort, the author measured that older fathers are less likely to produce embryos with an optimal number of blastomeres.[10] This effect was seen regardless of maternal age. It is known that ageing affects the integrity of germ cell DNA, but the author hypothesises that the sperm epigenome machinery may also encounter 'defects' due to ageing or due to paralleled effects from chronic exposure to a harmful environment throughout life.

Further research is warranted to better understand these observations. More epidemiologic studies are needed in other populations of males and future fathers. If this POHaD hypothesis can be confirmed, and a male's environment and age play an important role in his fertility and offspring health, it is important to inform clinicians. Patients need to be instructed about potential harm of their lifestyle (or age) to prevent development or worsening of health-related issues. Policy makers should inform the general public, increasing the awareness of potential consequences when delaying fatherhood, for instance. Furthermore, research is needed in the field of occupational epidemiology. It is yet unclear whether some occupational exposures in men before conception affect the sperm epigenome, and subsequently, offspring health.

CONCLUSION

In conclusion, the impact of the environment of future fathers cannot be ignored. Next to current precautions in pregnant females, the author believes there is an urgent need to include a male-focused strategy in future research and health policy plans. ■

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Hypnofertility for Reducing Stress and Increasing Fertility Preparedness

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Fertility, fertility preparedness, hypnofertility, reducing stress.

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BACKGROUND AND AIMS

Some studies indicate that in conditions of increased stress, oocyte development may be negatively affected.[1] Hence, endometrial blood flow and pregnancy outcomes are possibly adversely affected. In the light of this knowledge, the literature recommends that cortisol levels, an indicator of stress, should be checked before introducing assisted reproductive treatments and, if possible, treatment should be initiated once it reaches normal levels.[2] Decreasing the elevated cortisol levels may help females relax and improve their preparedness for treatment. Preparedness, as a concept, has been studied in terms of a patient's ability to perform self-care to change their lifestyle. It comprises of components such as the awareness phase, appraisal phase, and planning phase. Many healthcare providers explain that the failure of a patient's willingness to change or comply to a medical regimen as a lack of preparedness. Higher preparedness is associated with better treatment results.[3] One of these practices applied to females to reduce stress and increase fertility preparedness is hypnofertility. It is based on a strong and effective body-mind interaction and balancing the brain and body through positive words and thoughts.

MATERIALS AND METHODS

Hypnofertility claims that the mind strongly affects the body. and that fertility is a natural function. Functionally, it includes the conscious, subconscious, and critical hypnosis. The first one, consciousness, is favourably impacted by positive words (affirmations). Therefore, it aims to support the use of positive language in patients with fertility issues. For example, instead of saying that 'the eggs are not developed enough', it should be stated that 'eggs continue to develop'. Another critical component of this method is the subconscious, which can be positively influenced by imagination, visualisation, and relaxation techniques. These techniques are aimed at the imagination and the creation of positive visuals about fertility, besides relaxing the body. Conclusively, it is argued that the critical factors that make the individual limit themselves to the negative messages and receive only positive messages.

RESULTS

The Fertility Preparedness Scale, which contains affirmations used in hypnofertility, has been developed to evaluate fertility preparedness. It includes three sub-dimensions: hope and awareness, positive feelings and thoughts, and prepared body and brain. It is considered that when females read positive items, they can look in positive way forward fertility treatment.[4]

CONCLUSION

Hypnofertility is non-invasive, inexpensive, and easily applicable to use in a clinical routine. The use of this programme in care will provide a systematic, holistic, and knowledge-based approach to females. It is thought that the programme will be effective in reducing the stress of females, increasing the pregnancy outcomes, and changing the trust and perspective towards the nurse. For evaluating fertility preparedness, the Fertility Preparedness Scale can be used before or during fertility treatment. The scale is clinically feasible, short, and cost-free, which are crucial in the daily practice, both for professionals and patients. ■

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Interview

Jennifer Hall

Clinical Associate Professor and NIHR Advanced Fellow, University College London (UCL) Institute for Women's Health; Honorary Consultant in Public Health Medicine, UCL Hospital and Public Health England (PHE); Senior Epidemiologist, PHE, London, UK

Q1

What led you to undertake a PhD in Maternal Health and Epidemiology and ultimately pursue a career in reproductive health?

I've been interested in reproductive and maternal health since very early on in medical school. As an undergraduate, I did my dissertation on HIV and human rights. Similarly, as a public health registrar, I focussed on maternity- and reproductive health services-related work. There are several aspects of reproductive health that draw me to it but I think the main one is the social injustice aspect. The fact that most maternal deaths are preventable with known interventions leads us to conclude that society does not deem women's lives to be worth saving. It is shocking to me that so many women continue to die because of pregnancy and childbirth, which is a natural process and not a disease. I find it frustrating that we provide sub-standard services to women, where they are not treated with respect or dignity, and then turn around and wonder why women don't access services, which is one of the contributing factors to their morbidity and mortality. From a public health perspective, there are so many societal issues that affect women's health, such as access to education and services, types of employment, gender roles, and cultural and societal expectations. Many of these are outside the control of the individual, yet we place so much responsibility on women's shoulders for their own health and the health of their family. I want to try to bridge some of that gap and empower women to be able to make their own decisions, while shining a light on these structural determinants that also need addressing. A career as a public health academic is the way I hope to do that.

Q2

You were appointed the role of Senior Epidemiologist for Public Health England (PHE) last year as part of the response to COVID-19 pandemic. How has your typical working day changed as a result of the pandemic?

For a while it changed completely! I stepped away from my research and worked full-time for PHE on the day-to-day epidemiology of COVID-19 in the UK for 6 months last year. I am part of the team that produces the daily numbers of cases and deaths, and I oversaw the production of data for the weekly surveillance report. I also worked on specific projects, including HOSTED, which looks at household transmission and produced the first data on the impact of vaccination on transmission from vaccinated cases. This got a lot of media interest. Now, I am mostly back at my usual role of research and teaching at UCL but I still work a day a week for PHE on COVID-19 epidemiology, although not in the field of reproductive health.

"I want to try to bridge some of that gap and empower women to be able to make their own decisions, while shining a light on these structural determinants that also need addressing. A career as a public health academic is the way I hope to do that."

Q3

Your professional experience has involved travel to several destinations, such as Malawi and Myanmar. Where do you believe you gained the most experience and how have your experiences shaped who you are today and the successes that you have achieved?

I have been so lucky to spend time living and working in such diverse settings as Malawi, Myanmar, Honduras, and Chile, and they have all given me different experiences. It's hard to say where I gained the most because I was doing completely different things at different stages of my career. I think the opportunity to spend time living in a different culture is so valuable to help you start to understand how the culture you have grown up in shapes your thinking in ways that you don't realise. As a researcher, this is really important because our own world view shapes if and how we see problems, how we frame our research questions, and how we might answer them. What might seem, from the perspective of an outsider, to be a problem that needs fixing might be a perfectly rationale response when you understand the context.

Q4

You have worked extensively to improve health and social outcomes for women of reproductive age, in the UK and internationally. Could you comment on the on-going topic of socio-economical and racial disparities in reproductive health outcomes? What do you believe the solution to be?

This is such a big issue and, as I said, it is these inequities and the injustice of it that that I find so engaging. I wish I knew the solution! I think we have a lot more work to do to understand why these disparities exist and how to tackle them; however, I do not think that the solution lies solely within the health service. The disparities that we see in reproductive health outcomes have their roots in the wider determinants of health, although that doesn't mean that there aren't improvements that can be made in services. We have to tackle both.

Q5

What does the ongoing P3 (pregnancy planning, preparation, and prevention) study: "Assessing women's feelings and preferences regarding a future pregnancy" entail? What is this study aiming to achieve?

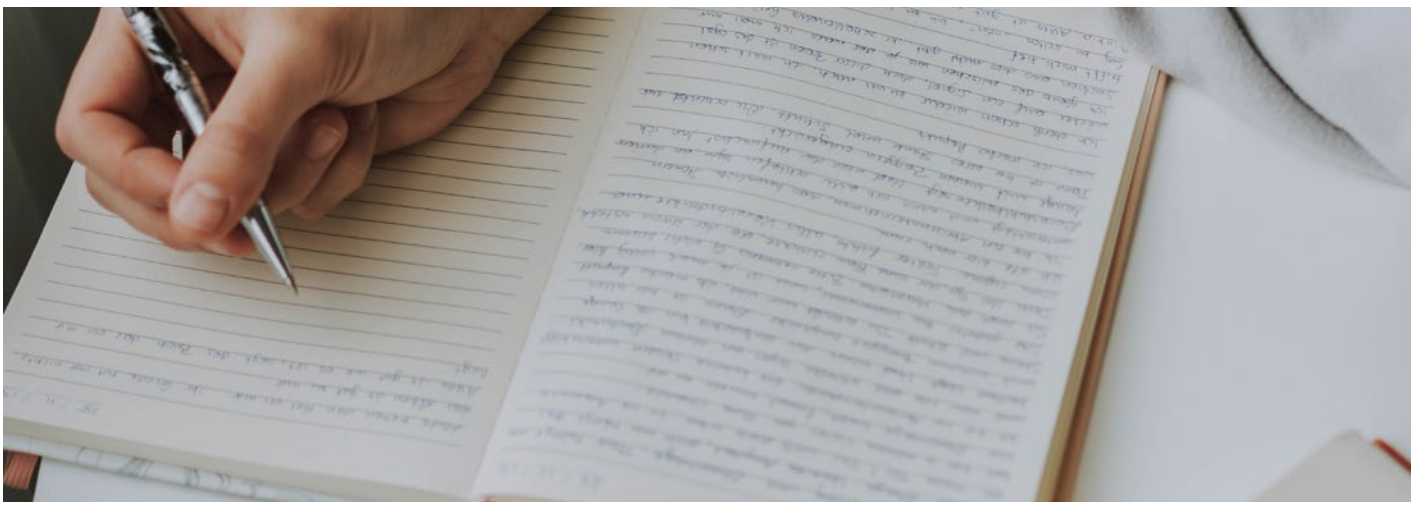
With this study, I am investigating how we can best support women to realise their reproductive goals, whatever they are. I am just as interested in helping women to avoid the pregnancies that they don't want as I am in helping them to

prepare for the pregnancies that they do want. However, our services in England are not set up in a way that healthcare professionals can support this. Not all nurses specialising in contraception feel skilled to discuss preconception health and not all midwives feel confident to have detailed discussions about postnatal contraception. Thus, in health service terms, women either access contraception services or antenatal care and, in the void in the middle, they do or don't get pregnant. This means we are missing a huge opportunity to improve preconception health to increase the chances of a healthy pregnancy for both mum and baby. The more we learn about the importance of the health of women, and men, around conception on the lifelong health and development of their children, the more urgent filling this gap becomes. Consequently, in this study, we are looking at different ways of identifying what women's intentions are for future pregnancies, if and how to incorporate it into primary care, and what the role of digital health, or 'FemTech', could be to support women, and their partners, to realise their reproductive goals. My aim is to see unplanned pregnancies coming down and women and men able to plan and prepare for the pregnancies they do want. I believe that this will have long-term societal benefits, including the reduction of non-communicable diseases.

"The disparities that we see in reproductive health outcomes have their roots in the wider determinants of health, although that doesn't mean that there aren't improvements that can be made in services."

Q6 As a mixed-methods researcher, where can we expect to see your focus lie in the coming years? Are there any advancements on the horizon for the field of reproductive health that you believe will be particularly noteworthy?

There has been a lot of interest in FemTech, with new apps to track periods, fertility, pregnancy, etc., and this has the potential to empower women to take control of their reproductive health. Although it is a little bit odd that, as one journalist found, if you forget to log your period in the app, then Facebook will start showing you adverts for baby clothes! However, this sort of technology is helping to dispel the myth that all women have a regular 28-day cycle, with ovulation occurring on Day 14, because the volume of data collected with these apps is a phenomenal source of information. My focus over the next few years is going to be in normalising conversations about pregnancy planning, both with health professionals and between individuals, because, as participants in our research reflected, why should it be taboo? There are so many hugely important aspects of women's health, like miscarriage or menopause, that are not openly talked about yet have a major impact on women's lives. Clearly, this needs to be addressed.



Q7 What advice would you give to expectant mothers during the COVID-19 pandemic in terms of pregnancy planning and preconception care?

I can only imagine how hard it has been to be pregnant during the uncertainly of the COVID-19 pandemic and for women who haven't had the maternity leave that they had envisaged, or who have had their fertility treatment delayed, or who haven't been able to access contraception services. I'd urge all women (and men) who are thinking about having a baby, pandemic or not, to look at how they can optimise their preconception health (e.g., by taking folic acid, achieving a healthy weight, stopping smoking, or reviewing any medications they are on). This is good for your own health, but also for the chances of a healthy pregnancy and baby. You need to build in time for that before you start trying because one in three couples will get pregnant in the first month of unprotected sex, so make sure you are using effective contraception until you are ready. Everyone should get the vaccine when they are offered it. If you can, I would have it before getting pregnant; however, if you are already pregnant and are offered the vaccine, then discuss with your healthcare provider what your options are and which is the best vaccine for you. Also, if you are pregnant, think ahead to what you would like to use for postnatal contraception because it might be easier to have this sorted at delivery (e.g., have a coil put in) rather than try to access these services later, especially if there are more lockdowns but it can be tricky even just with a newborn baby! Above all, be kind to yourself. Pregnancy and the postnatal period is a tough (and wonderful) time, made all the harder by the pandemic as it has taken away our 'village'. Therefore, make sure you get the support that you need.

The Use of Frozen Embryo Transfer and the Development of Pregnancy-Induced Hypertension: A Literature Review

EDITOR'S PICK

The Editor's Pick for this publication is the interesting review by Agbabiaka and D'Angelo, who examine the use of frozen embryo transfers (FET) and the development of pregnancy-induced hypertension. The use of frozen embryos in assisted reproduction was successfully introduced some years ago, with good outcomes in terms of pregnancy rate and live birth. However, whether pregnancy-induced complications such as hypertension and pre-eclampsia are different respect to fresh transfers is less clear. The authors, after reviewing the studies that report the incidence of hypertension and pre-eclampsia after FET, evidence a possible relation between FET and hypertension, with possible implications for clinical practice.

Elisabetta Baldi

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Abstract

The use of assisted reproduction techniques has been associated with obstetric complications. An understanding about which methods and treatment protocols produce better outcomes would provide greater opportunities for a successful pregnancy. The aim of this literature review was to identify whether frozen embryo transfer (FET) leads to a greater incidence of pregnancy-induced hypertension (PIH) compared to fresh embryo transfer. Fifteen studies were identified and subsequently reviewed. Eleven studies suggested FET increased the incidence of PIH–gestational hypertension and pre-eclampsia. The evidence suggests a correlation between FET and PIH. Exploration into why this is the case should be the focus of future studies. Implications for clinical practice involve extensive preconception counselling and potentially advising prophylactic low-dose aspirin with the aim of lower the incidence of PIH.

INTRODUCTION

Subfertility affects 1/6–7 couples worldwide.[1] Assisted reproduction techniques such as *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) are treatment options for these couples. Hypertension-related disorders affect 8–10% of pregnancies in the UK.[2] It can cause fatal consequences, such as premature labour and intrauterine growth restriction, and maternal consequences, e.g., liver damage and stroke.[3] Using fresh or frozen embryos are both options for IVF/ICSI under the National Institute for Health and Care Excellence (NICE) guidelines.[1] The Human Fertilisation and Embryology Authority (HFEA)[4] reports approximately one-third of IVF/ICSI cycles result in frozen embryos. It would be beneficial to better understand the effect of frozen embryo transfer (FET) related to obstetric outcomes so that optimal care can be provided. A literature review was performed to assess the current evidence to determine whether FET leads to a greater rate of pregnancy-induced hypertension (PIH), e.g., pre-eclampsia (PE) and gestational hypertension (GH). The objective of this review was to identify whether FET use in IVF/ICSI leads to a greater incidence of PIH, specifically, PE and GH, compared to when fresh embryo transfer (ET) is used.

METHOD

Objective

To identify whether FET use in IVF/ICSI leads to a greater incidence of PIH, specifically PE and GH, compared to when fresh ET is used.

Outcomes

The primary outcome was the development of PIH, to include GH and PE.

A Medline search was performed from inception until April 2020. The following search terms were used: "assisted reproductive techniques", "assisted reproductive treatment", "assisted reproductive technology", "*in vitro* fertilisation", "IVF-ET", "embryo transfer", "intracytoplasmic sperm injections", "oocyte donation", and "sperm donation" alongside "pre-eclampsia", "pregnancy induced hypertension", "gestational hypertension", and "eclampsia", alongside "cryopreservation", "vitrification", "fresh embryo", "frozen embryo", "frozen blastocyst", and "blastocyst vitrification".

Sixty articles were found and screened by reading the title and abstracts. Studies that compared the use of FET and fresh ET and the development of PIH were selected. Inclusion criteria for the selected studies were English-language papers, human studies, randomised controlled trials (RCT), cohort studies, case-control studies, retrospective, and prospective studies. Exclusion criteria were systemic reviews/meta-analysis, letters to the editor, and case reports/abstracts proceedings (grey literature). Nineteen articles were included in the final analysis. The screening process is summarised in Figure 1.

RESULTS

Following the search, 19 articles were retrieved in full, and four were excluded because they did not have the appropriate data or analysis comparing FET and fresh ET (Figure 1). Fifteen articles fulfilled the inclusion criteria. Tables 1 and 2 outline the study type, main results, and comments about the included articles. Table 1 includes articles relating to PE and GH as separate outcomes.

Table 2 includes articles relating to PIH and hypertensive disorders in pregnancy as a single outcome. PIH includes GH, PE, and eclampsia. Hypertensive disorders in pregnancy refer to conditions associated with PIH, as well as pre-existing hypertension complicating pregnancy.

Of the 15 articles reviewed, summarised in Tables 1 and 2, a majority (11/15) of papers found that FET confers a higher risk of developing PIH.[6-9,13-19] Chen et al.[7] and Wei et al.[13] found an increased incidence of PE but not GH in the FET group. After adjusting for maternal age, BMI, parity, multiple gestations, and smoking, excluding donor oocytes, the results were consistent (Tables 1 and 2). Imudia et al.[5] found that fresh ET conferred a higher risk of PE compared to FET.

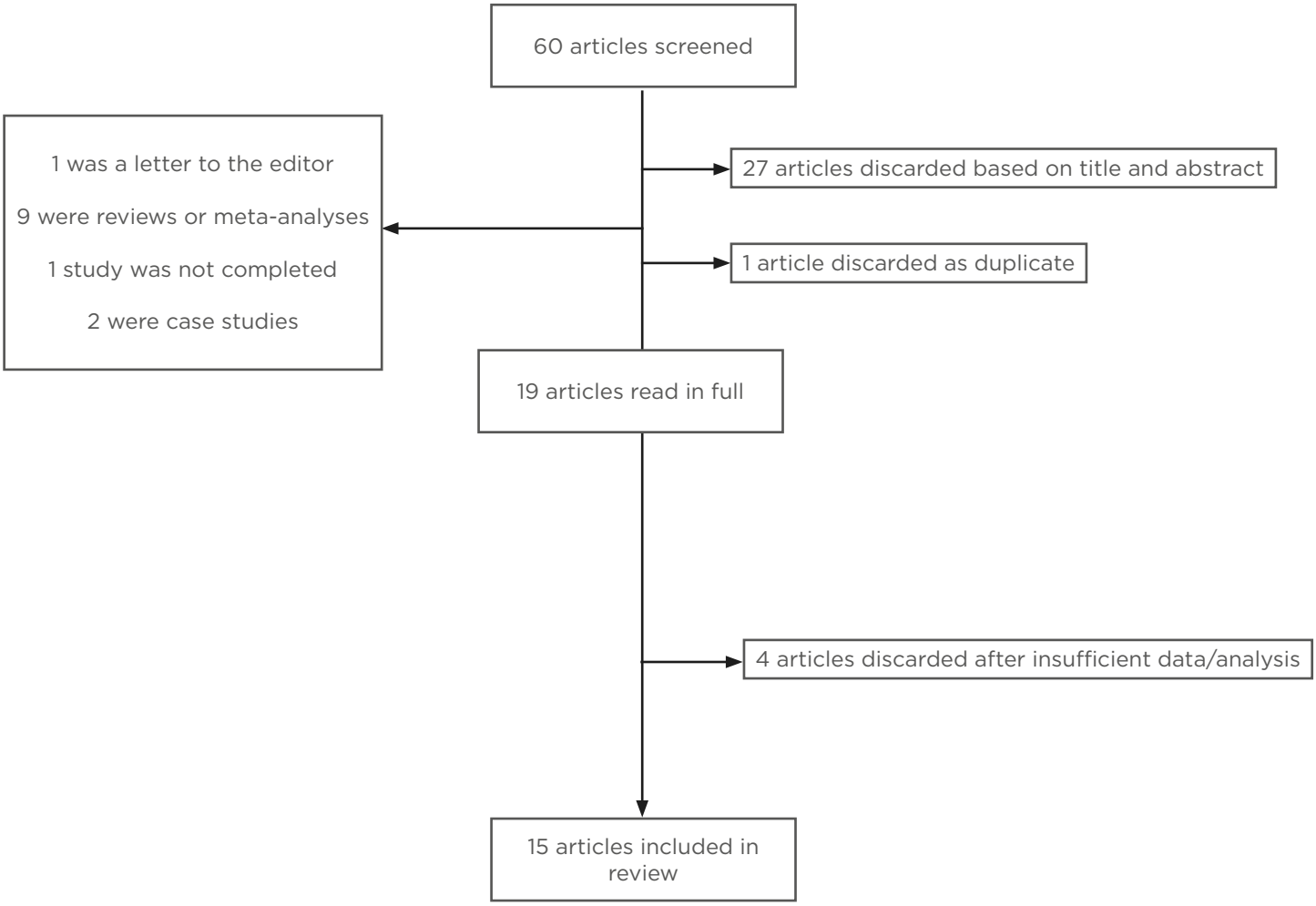

Figure 1: Screening process of articles after keyword search.

Table 1: Study characteristics and incidence of pre-eclampsia and gestational hypertension.

Publication	Study design	Population (fresh versus frozen)	HRT or natural cycle	Outcome measure	Results (95% CI)	Comments
Imudia et al.[5]	Retrospective single-centre cohort study	32 versus 20	HRT	PE	22% (fresh) versus 0% (frozen); p=0.04	Only singleton pregnancies and patients with high peak oestrogens. Adjusted for BMI, antral follicle count, and peak serum oestrogen level

Table 1 continued.

Publication	Study design	Population (fresh versus frozen)	HRT or natural cycle	Outcome measure	Results (95% CI)	Comments
Barsky et al.[6]	Retrospective single-centre cohort study	289 versus 109	HRT	PE	aOR: 3.1 (1.2–8.4); p=0.023	Adjusted for maternal age, newborn sex, diabetes status, and parity. Singleton pregnancies and autologous oocytes only
Chen et al.[7]	Multicentre RCT	762 versus 746	HRT	GH	Rate ratio: 1.97 (0.68–5.71); p=0.20	Exclusively patients with PCOS. Adjusted for study site and baseline characteristics, e.g., maternal age, BMI
				PE	Rate ratio: 3.12 (1.26–7.73); p=0.009	
Jing et al.[8]	Retrospective single-centre cohort study	129 versus 188	Not specified	GH	aOR: 4.85 (1.34–17.46)	Adjusted for maternal age, BMI, parity, twins, neonatal sex, and number of embryos transferred. FET versus fresh ET not the only variable being studied; also comparing stage of embryo biopsy. FET with blastocyst biopsy, and fresh ET with cleavage biopsy
Sites et al.[9]	Retrospective multicentre cohort study	13,976 versus 1,961	Not specified	PE	Singleton and autologous eggs aOR: 2.17 (1.67–2.82); p<0.0001	Adjusted for BMI (missing >50% of the data), maternal age, maternal race, diabetes, parity, HTN, infant sex, and birth year. Unadjusted p values with results concerning GH, twins, and donor oocytes

Table 1 continued.

Publication	Study design	Population (fresh versus frozen)	HRT or natural cycle	Outcome measure	Results (95% CI)	Comments
Xiong et al.[10]	Single-centre retrospective cohort study	446 versus 212	Both	GH	OR: 0.44 (0.13–1.34); aOR: 0.49 (0.15–1.51)	Adjusted for maternal age, BMI, and infertility years
				PE	OR: 0.93 (0.42–1.96); aOR: 0.98 (0.44–2.12)	
Blazquez et al.[11]	Retrospective single-centre cohort study	353 versus 80	HRT	GH	aOR: 1.45 (0.75–2.81); p=0.27	Only donated oocytes. Adjusted for maternal age, BMI, primigravity, and multiple pregnancy
				Preterm PE	aOR: 1.95 (0.72–5.26); p=0.18	
				Term PE	aOR: 0.30 (0.04–2.35); p=0.25	
Lei et al.[12]	Retrospective single-centre cohort study	1,583 versus 673	Not specified	GH	10.2% (fresh) versus 10.0% (frozen)	Autologous eggs. No aOR provided for fresh ET versus FET. No p value provided for fresh ET versus FET
				Mild PE	3.3% (fresh) versus 3.0% (frozen)	
				Severe PE	1.3% (fresh) versus 1.3% (frozen)	
Wei et al.[13]	Multicentre RCT	401 versus 512	Both	GH	RR: 1.27 (0.53–3.04); p=0.59	Extensive exclusion criteria. Singleton pregnancies only
				PE	RR: 3.13 (1.06–9.30); p=0.029	

aOR: adjusted odds ratio; ART: assisted reproduction technique; CI: confidence interval; ET: embryo transfer; FET: frozen embryo transfer; GH: gestational hypertension: HTN: hypertension; HRT: hormone replacement therapy; OR: odds ratio; PCOS: polycystic ovarian syndrome; PE: pre-eclampsia; RCT: randomised controlled trial; RR: relative risk.

Table 2: Study characteristics and incidence of pregnancy-induced hypertension and hypertensive disorders in pregnancy.

Publication	Study design	Population (fresh versus frozen)	HRT or natural cycle	Outcome measure	Results (95% CI)	Comments
Ishihara et al.[14]	Retrospective nationwide cohort study	18,478 versus 34,545	Not specified	PIH	aOR: 1.58 (1.35–1.86); p<0.001	Only autologous oocytes and singleton pregnancies. Adjusted for maternal age and sex of infant
Opdahl et al.[15]	Retrospective multi-country cohort study	49,560 versus 7,493	Not specified	Hypertensive disorders in pregnancy	Singleton aOR: 1.24 (1.11–1.37) Twins aOR: 1.72 (1.45–2.04) Same mother OR: 1.60 (1.12–2.28); aOR: 2.63 (1.73–3.99)	Adjusted for parity, maternal age, birth year, country, and offspring sex. Able to compare FET versus fresh ET in same mother
Belva et al.[16]	Retrospective single-centre cohort study	1,517 versus 912	Natural cycle (majority)	PIH	7.2% (fresh) versus 13.4% (frozen); p=0.0001	Adjusted for number of embryos transferred, embryo stage at transfer/day of transfer, maternal age (significantly higher in vitrified group), BMI, parity, and smoking
Ginström Ernstad et al.[17]	Retrospective multicentre study	24,365 versus 9,726	Both	PIH	OR: 1.33 (1.21–1.47); aOR: 1.51 (1.35-1.68)	Adjusted for maternal age, BMI, parity, year of birth, smoking, chronic HTN, infant sex, maternal education, cause of infertility, years of infertility, freezing method, culture duration, ART method, and number of gestational sacs. Singleton pregnancies. Autologous oocytes only.

Table 2 continued.

Publication	Study design	Population (fresh versus frozen)	HRT or natural cycle	Outcome measure	Results (95% CI)	Comments
Ginström Ernstad et al.[18]	Retrospective cohort multicentre study	4,459 versus 3,659	Not specified	PIH	OR: 1.39 (1.16–1.67); aOR: 1.47 (1.19–1.81)	Adjusted for country, year of birth, maternal age, BMI, parity, smoking, number of gestational sacs, parental education, ART method, and child sex. Singleton pregnancies. Autologous oocytes only
Luke et al.[19]	Retrospective multicentre cohort study	62,192 versus 21,390	Not specified	PIH	Autologous oocytes aOR: 1.30 (1.20–1.40) Donor oocytes aOR: 1.70 (1.47–1.96)	Adjusted for BMI, maternal age, race/ethnicity, parity, education, country, state, year of birth, infant sex, and cause of infertility. Singleton pregnancies only

aOR: adjusted odds ratio; ART: assisted reproduction technique; CI: confidence interval; ET: embryo transfer; FET: frozen embryo transfer; HTN: hypertension; HRT: hormone replacement therapy; OR: odds ratio; PIH: pregnancy-induced hypertension; RR: relative risk.

DISCUSSION

The results suggest that FET increases the incidence of PIH. A variety of studies display this correlation, including RCTs. Each study focussd on different elements and populations giving validation to this claim. The main limitations of these results are the quality of the evidence presented.

Risk Factors of Pre-eclampsia

When looking at the evidence presented, the impact of confounders on the results must be considered, such as risk factors for PE, which include older age, multiple gestation, BMI, past medical history (PMH) of PE, diabetes, family history of PE, and parity.[20,21]

Most of the studies had information on risk factors and adjusted their results accordingly (Tables 1 and 2), finding consistent results. However, most of the studies did not adjust for PMH,[5-12,14-19] which is a significant risk factor for PIH. Bartsch et al.,[20] who conducted a meta-analysis of over 90 studies, found that PMH of PE increased the risk of developing PE in subsequent pregnancies by eight times. The same study found that pre-gestational diabetes almost quadrupled the risk of developing

PE.[20] Nonetheless, the studies that adjusted for or excluded pre-gestational diabetes still found that FET increased the risk of developing PIH.[6,9,19]

IVF/ICSI is a complex process with many variables that may factor into the development of PIH. This information was not always accounted for.[6,9,10,14,15,17-19] Embryo biopsy for pre-implantation genetic testing and donor oocytes have been associated with significantly increased risk of PIH.[22-24] However, studies excluding donor oocytes still had an increased risk of PIH during FET.[6,9,14,17-19]

Natural versus Hormone Replacement Therapy Cycle in Frozen Embryo Transfer

A potential factor in developing PIH during FET is the type of endometrial preparation used for the FET cycle: natural versus hormone replacement therapy (HRT). Of the 15 articles reviewed, eight commented on the method used for endometrial preparation (Tables 1 and 2). This is a confounding factor that most studies have not adjusted for (Tables 1 and 2). A specific analysis comparing HRT versus natural cycles in FET found that HRT cycles lead to an increased risk of PIH compared to natural cycles, adjusted odds ratio 1.78,[17] suggesting a role for HRT in the development of PIH.

The mechanism by which HRT cycles, commonly used in FET, increase the risk of PIH is still debated. A suggested mechanism is the absence of the corpus luteum (CL), a feature of HRT cycles; subsequently, the absence of hormones produced by the CL could adversely affect the maternal cardiovascular system.[25,26] von Versen-Höynck et al.[26] found that HRT with no CL had a significantly higher incidence of PE compared to natural FET with one CL.

Study Type

The study type will contribute to the quality of the evidence. The majority of the papers were retrospective cohort studies (Tables 1 and 2). One of the major limitations is that information on confounding factors may not be available. Another disadvantage is that any missing information deemed important would be difficult to find and follow up. However, retrospective cohort studies have the advantage of low risk of response bias because individuals did not know what the data would be used for, and large sample sizes, which allow studies to be more representative of the general population.

The highest-quality evidence comes from RCTs. RCTs reduce the risk of selection bias because participants are randomly assigned to each group. Unfortunately, these trials were not double-blinded, and in this circumstance, may not be ethically possible. RCTs are able to control a variety of potential confounders. Both of the RCTs found an increased risk of PE when FET was used, but GH incidence was not affected.[7,13]

Multicentre studies have the added benefit of reduced selection bias because of the wider range of participants available, and allow conclusions to be made with regard to a larger patient base (Tables 1 and 2). However, there is a risk of performance bias because the patients are more likely to have differences in their prenatal/antenatal care and treatment. Some of the studies had a wide date period of 10+ years.[15,17-19] This leads to an extensive sample size, reducing selection bias; however, it can expose the study to unknown confounders because medical practices and lifestyle can change during that time period.

A Nordic study[15] found in the same mother the chance of developing PIH was increased by 2.5 times when using FET compared to fresh ET.[15] This is strong evidence because many maternal confounding factors can be removed with this model, suggesting the correlation between FET and PIH is not solely due to maternal factors. However, the sample size for this analysis was small.

All four of these papers that did not see a correlation between PIH and FET are single-centre studies.[5,10-12] The advantage is that there is a reduced risk of confounders associated with variation of prenatal/antenatal care and guidelines affecting the results. However, they are more likely to have smaller sample sizes.[5,11] The four studies had an increased risk for Type 2 error, where a difference in treatment outcome is not seen but there is one present, because of a significantly smaller sample size in the FET versus fresh ET group. Blazquez et al.[11] had a wide confidence interval, which makes it difficult to interpret where the true odds lie. The evidence provided by Lei et al.[12] is limited because of a lack of statistical data, i.e., the p value.

Previous Studies

Previous reviews and meta-analyses have also found an increase of PIH in patients with FET.[27-29] Maheshwari et al.[27] included five studies in their meta-analysis and found FET led to a 29% increased risk of PIH (relative risk [RR]: 1.29). Similarly, Sha et al.[28] reviewed seven studies and found a 44% increased risk of developing PIH when using FET (RR: 1.44) in relation to PIH development. However, these reviews also showed a lower risk of other obstetric complications, e.g., preterm delivery, placenta praevia, and placental abruption.[27,28] This suggests there is a role for FET and that special considerations should be taken when comparing the options.

Clinical Implications

Clinically, these data suggest a possible use of preventative treatment, e.g., low-dose aspirin during FET in high-risk individuals. The efficacy of aspirin in preventing PE has been replicated in >70 trials and reduces the risk of developing PE by 18% (RR: 0.82).[30]

NICE[31] recommend that patients with risk factors take aspirin daily from the 12th week of gestation. Based on the evidence presented, there may be a role for including FET as a risk factor. Compared with other risk factors, such as PMH or family history of PE, the chance of developing PE with FET may not be increased as dramatically. However, in the setting of assisted reproduction techniques, where there are often limited chances, it seems worth investigating a role for prophylaxis. Clinicians should consider their use of FET when weighing up the risk of PIH and monitor patients more closely.

CONCLUSION

This literature review comparing the effect of FET and fresh ET on development of PIH shows there is an association between FET and PIH. There was a large number of articles[15] reviewed, including two RCTs, compared with previous reviews, which included only 2–7 articles and were limited to only observational studies.[27-29] This review could highlight a role for use of preventative treatment during FET in high-risk individuals. It also suggests the importance of providing appropriate counselling during the FET cycle and potential referral for antenatal counselling when other risk factors are associated. This is the first review to discuss the potential use of aspirin in FET. Though there is a correlation established, future studies exploring the reasons for FET conferring a greater risk are needed. This could be due to embryo quality following the cryopreservation and thawing procedures or different intrauterine environment, e.g., absence of the CL,[17,25] or ultimately, to the hormonal treatment and protocols associated with FET.[15]

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Uterine Incision at the Fundus, Transitory Devascularisation, and Myometrial Resection for Uterine Preservation in Anterior Accretism: A Case Report

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Abstract

Placenta accreta spectrum is a serious obstetric condition related to abnormal adherence of placental tissue to the myometrium and high maternal and fetal morbidity. In order to achieve the best outcome, the management of this condition must be carried out by an experienced multidisciplinary team and the individual characteristics of the patient must be taken into consideration, such as comorbidities and desire for reproductive preservation. This case report presents the conservative surgical management of placenta accreta spectrum in a 23-year-old patient who underwent an elective caesarean section with uterine preservation because of anterior placenta increta. The authors performed a transverse uterine incision at the fundus with transitory uterine devascularisation of the lower uterine segment with partial myometrial removal. This technique was successful for controlling the haemorrhage and preserving the uterus, with no complications.

INTRODUCTION

Placenta accreta spectrum (PAS) is a set of obstetric conditions with abnormal adhesion of placental tissue to the myometrium. These conditions differ according to the degree of trophoblastic invasion to the myometrium: attachment to the myometrium (placenta accreta), invasion into the myometrium layer (placenta increta), or invasion through the myometrium to reach the serosa and other adjacent structures (placenta percreta).[1] PAS is associated with high maternal and fetal morbidity and mortality, particularly because of the risk of major haemorrhage, requirement of haemotransfusion, peripartum hysterectomy, and prematurity.[2] The haemorrhage is related to the depth of myometrial invasion, the size of the

adhesion area, and the presence of invasion of adjacent structures by the placenta.[3]

In recent years, the most common interventional approach for PAS has been elective caesarean section (C-section) followed by hysterectomy;[3] however, conservative interventions can also be used, while taking into account the reduction of unfavourable outcomes, such as bleeding and the subsequent complications.[4] In this article, the authors report a case of a patient with anterior placenta increta who underwent elective C-section with uterine preservation. The technique applied was uterine incision at the fundus, with transitory uterine devascularisation with partial myometrial removal.

CASE REPORT

A 23-year-old patient, gravida 2, para 1 (one previous C-section), with no comorbidities, was monitored in a low-risk prenatal care setting from 11 weeks' gestation. She had no history of any surgical procedures (apart from the C-section), uterine manipulation, smoking, or previous pelvic infection.

The patient arrived at the maternity ward emergency room of a high-complexity hospital at 38/39 weeks' gestation (dated from the last menstrual period, accordant with the first ultrasound at 11/12 weeks), presenting prior obstetric ultrasonography results performed in an outpatient service, which indicated an anterior increta placenta. New ultrasonography (transabdominal and endovaginal) was performed by specialists. The results showed an anterior placenta with low segmental insertion (and not reaching the internal orifice of the uterine cervix). It also revealed, on the right area of low segment, a partial loss of myometrial continuity, increased vascularisation on Doppler ultrasound findings, and a healthy fetus.

On examination of the patient's medical records from prenatal care, it became apparent that a diagnosis of anterior placenta accreta was already suspected from the first performed ultrasound. This detailed a placenta with anterior topical insertion, presenting inaccurate limits and irregularities with the myometrium and prolongations of its basal vessels penetrating into the uterine wall using Doppler. In another ultrasound performed at 12/13 weeks' gestation, a placenta of anterior insertion was reported, which covered the internal orifice of the cervix, without other changes. The next ultrasound was performed at 31/32 weeks' gestation and the abnormality was neither described nor investigated.

The patient was only advised about the diagnosis when entering the high-complexity hospital. The patient expressed her desire for future pregnancy, which led the surgical plan to attempt a conservative technique. The patient was informed about the higher risk of uterine rupture if future pregnancy was to occur after the procedure, in addition to the risk of major haemorrhage and the possibility of having to perform a hysterectomy during surgery. A multidisciplinary team constituting gynaecological surgeons, obstetricians, anaesthesiologists, and neonatologists were responsible for the surgical plan. In the surgical plan, the team considered how the ultrasound scans suggested placenta increta in the right lower uterine segment, with no signs of other organ involvement and an absence of signs of praevia or low-lying placenta. It was planned to perform fetal extraction through a transverse incision in the uterine fundus. In order to reduce the risk of haemorrhage, the use of a Penrose drain for uterine reversible devascularisation was considered. The resection of the area of placental invasion would be performed in the absence of intra-operative signs of placenta percreta, from the exposed myometrium with the transverse incision. Laboratory tests were checked, as well as blood components, and an intensive care unit was reserved because of the high risk of major haemorrhage.

Pre-operative haemotransfusion was performed because the patient had a haemoglobin count of 8.5 mg/dL. The procedure occurred under spinal anaesthesia with bladder catheterisation. The anaesthesia team was prepared to alter the procedure to general anaesthesia in case of haemodynamic instability. A median xipho-pubic incision was chosen for better uterine exposure and exteriorisation because of advanced gestational age and the risk of requiring extensive adhesiolysis. The upper placental border was identified by palpation and a unique transverse incision at 3 cm from the uterine fundus was performed. The fetal extraction was pelvic, with a first-minute Apgar score of 9. A Penrose drain

was used for tying the uterine segment, including the uterine and ovarian arteries with reversible and transitory uterine devascularisation (Figure 1). After the placenta was extracted, placenta increta was visible in the uterine anterior wall; the uterine segment showed invasion of <70% of myometrial layer from gross visual inspection.

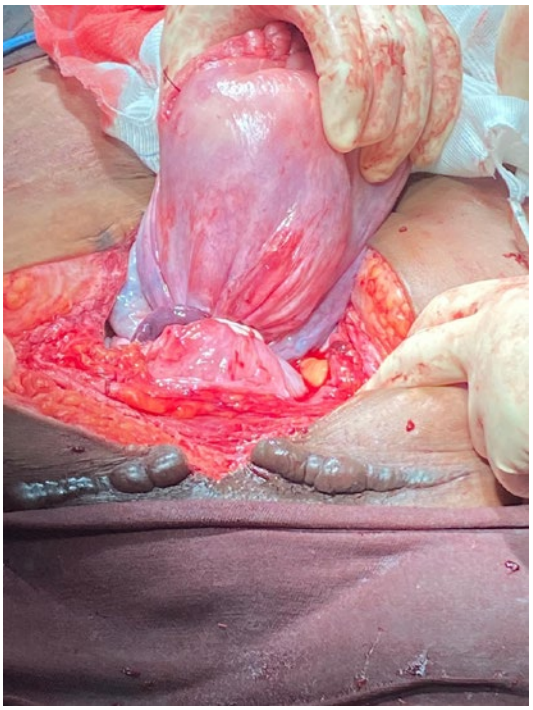

Figure 1: Penrose drain reducing vascular flow in uterine and ovarian arteries.

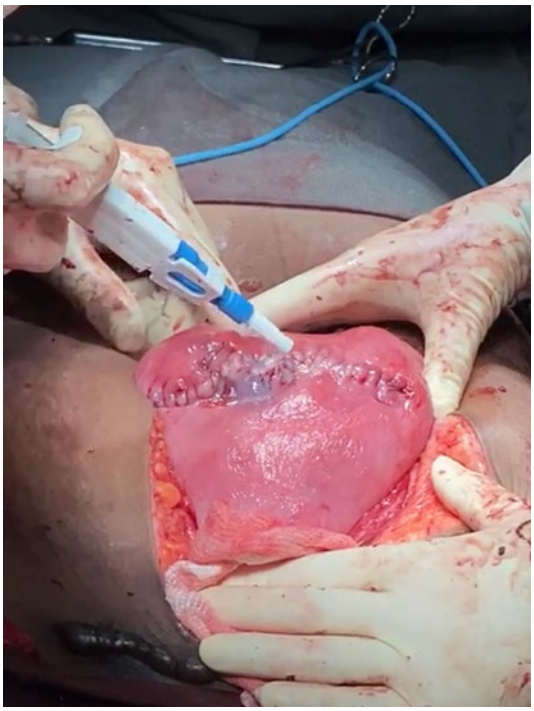

Figure 2: Application of fibrin glue on two-plane hysterorrhaphy.

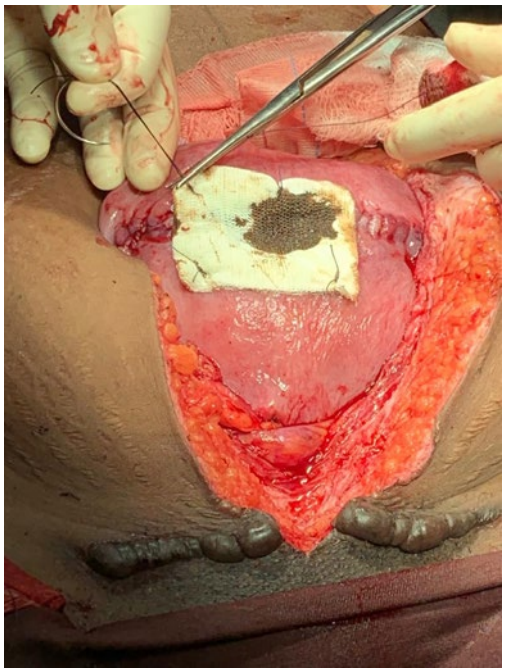

Figure 3: Application of oxidised regenerated cellulose.

Longitudinal resection of the myometrial area with placental invasion was performed: about 10 cm longitudinally and 4 cm vertically, covering the entire thickness of the uterine wall. It was sutured for internal recomposition of the uterine segment and hysterorrhaphy, in a double-layer suture. The uterine segment was untied and uterine revascularisation was observed. Haemostasis was reviewed, followed by application of fibrin glue and oxidised regenerated cellulose (absorbable haemostat) over the hysterorrhaphy (Figures 2 and 3). There were no signs of placental invasion on bladder or ureteral lesions. The patient was stable and did not require further haemotransfusion or intensive care. She was referred to the obstetrics and gynaecology ward for clinical follow-up; here, she was discharged after 72 hours and referred for outpatient follow-up. She was advised about the need for high-risk prenatal follow-up in the case of subsequent pregnancies, as well as the possibility of recurrence of placenta accreta and uterine rupture.

DISCUSSION

Although the pathophysiology of PAS is not completely understood, it is known to be related to the abnormal decidualisation process, with abnormal placental invasion and adherence within the uterine scar tissue as a result of an endometrial–myometrial interface defect.[5] The two main risk factors are previous C-section and placenta praevia in current pregnancy,[6] and the risk is increased when both factors are present[1] or by a higher number of C-sections.[7] PAS has an estimated prevalence of between 0.01% and 1.1% in the general population[8] and there has been a significant increase in its incidence in the last 40 years, mainly as a result of high C-section rates.[1]

Approach to PAS should be performed by an experienced multidisciplinary team, preferably through an elective C-section or hysterectomy, between 34 weeks and 35 weeks and 6 days of gestational age (degree 1A of evidence).[1] If possible, the diagnosis should be made in the prenatal stage by ultrasonography or MRI in order to allow for adequate planning.[1,9,10] Some image findings (ultrasound or MRI) have been related to PAS, such as multiple placental lacunae (intraplacental sonolucent spaces), loss of the hypoechoic area behind the placenta, loss of the line representing the bladder wall–uterine serosa, myometrial thinning, abnormal vascularity, and uterine bulging into the bladder.[11] The conservative strategy includes procedures that

aim for uterine preservation, not only owing to the risks of a peripartum hysterectomy but also for the desire to preserve fertility. These conservative approaches essentially leave the placenta *in situ* to wait resorption, which should occur between 4 weeks and 12 months, and resect the invaded tissue with the placenta.[4,12] However, potential complications include infection, intra-operative bleeding, and the need for hysterectomy.[4,12,13] Adjuvant treatment with methotrexate is not recommended because of adverse effects.[4,12]

Since the report of the 'one-step conservative surgery' for placenta accrete in 2004, which consists of resection of invaded tissue and myometrial reconstruction, other approaches have been described for bleeding control, including vascular interruption of newly formed vessels, bilateral uterine artery ligation, and iliac balloon insufflation. Resection of the area of placental accretism with myometrial repair is considered appropriate when the anterior defect involves up to 50% of the uterine circumference. Fibrin glue and oxidised cellulose are useful in the control of bleeding and haemostasis.[14]

In 2019, a study was published that described and evaluated the technique of parallel transverse uterine incisions, which consists of a transverse incision at the uterine fundus for fetal extraction and a segmental transverse incision above the upper border of the placenta for resection of the invaded myometrium. This is followed by pelvic devascularisation by tourniquet with two rubber tubes: the first is tightened to the lower uterine segment to restrict uterine flow and the second is connected to the uterine body to restrict ovarian flow, along with insufflation of occlusion balloons in internal iliac arteries. The study showed significant statistical differences regarding blood loss, need for haemotransfusion, conversion to hysterectomy, and admission to the intensive care unit.[13]

The technique of single uterine incision at the fundus with transitory uterine devascularisation using the Penrose drain, tightly ligating the lower uterine segment and ovaries for concomitant compression of uterine and ovarian arteries, represents a promising adaptation of the myometrial resection and reconstruction techniques. The technique is efficient and allows for bleeding control with less-complex surgical techniques. The single transverse uterine incision at the fundus reduces bleeding foci and provides more time to control bleeding in emergency situations, including ligation of uterine arteries or insufflation of pelvic vascular balloons, as in the case of intra-operative diagnosis of PAS. In addition, a single transverse incision provides fewer foci of fragility in the uterine wall, which likely reduces the risk of uterine rupture in subsequent pregnancies compared to techniques in which more than one incision is performed.

Proper planning and appropriate patient selection are essential. The technique can be used on patients with anterior placenta accreta, in which the area of accretism does not compromise >50% of the uterine circumference, in addition to the absence of signs of placenta percreta. All patients should be thoroughly informed about the intra-operative risks of the procedure, in addition to the risks of uterine rupture in case of new pregnancy. The team must be prepared for potential complications and the possibility of switching from the conservative procedure to an obstetric hysterectomy.

CONCLUSION

Although hysterectomy is the standard treatment for PAS in many countries,[12] conservative techniques, especially accretism resection with myometrial repair, are excellent options for selected cases. The choice of treatment should consider not only the surgeon and patient's preference but also the resources and circumstances of the approach. The authors advise that all patients who are candidates for the procedure of uterine incision at the fundus with transitory uterine devascularisation or other conservative techniques should be thoroughly informed regarding the risks of the procedure. These include risk of major intra-operative haemorrhage and the risk of recurrence of placenta accreta, in addition to higher risk of uterine rupture by conceiving a subsequent pregnancy in a uterus that has had the lower uterine segment and part of the anterior uterine wall with myometrium approaching the uterine fundus removed. Prospective studies with multiple cases of the uterine incision at the fundus, with transitory uterine devascularisation with myometrial resection, are important to evaluate the applicability and to compare with other techniques already consolidated.

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Examining Diagnostic Options and Classification Systems Available for Endometriosis

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Abstract

Introduction: Endometriosis is characterised by the presence of endometrium-like tissue outside the uterus, and is often associated with chronic pelvic pain, infertility, and compromised quality of life. Development of reliable methods of early diagnosis, staging, and classification of endometriosis would allow for restriction of disease progression by its early detection and strategising towards its early treatment and management.

Diagnostic options: Typically, diagnosis and staging of endometriosis include a history and physical examination followed by clinical, imaging, and laparoscopic findings. Surgical inspection of lesions at laparoscopy with histological confirmation remains the most reliable procedure towards the detection of endometriosis and its classification. Although there are many putative peripheral biomarkers having potential diagnostic values for endometriosis, further studies are necessary for their validation.

Classification systems: Based on anatomical, clinical, imaging, and several pathophysiological findings, various classifications and staging systems of endometriosis, e.g., revised American Society for Reproductive Medicine (rASRM), ENZIAN, Endometriosis Fertility Index (EFI) and Foci–Ovarian endometrioma–Adhesion–Tubal endometriosis–Inflammation (FOATI) scoring systems, have so far been postulated. However, there is no fool-proof diagnostic and classification approach available for the disease due to the general failure of current systems to reflect reproducible correlation with the major symptoms of endometriosis.

Conclusion: A 'toolbox approach', using all the available diagnostic and classification systems maximising the information available to healthcare providers and females, is a recent recommendation. Development of collaborative research networks for the harmonisation of patient information, biological sample collection, and its storage, and that of methodological and analytical tools in a wider patient base is necessary to discover reliable leads for future diagnostic options and a classification system for endometriosis.

INTRODUCTION

Endometriosis is a complex gynaecological disorder characterised by the presence of endometrium-like tissue outside the uterus, primarily on pelvic organs, and affects approximately 10% of females of reproductive age.[1] One out of two patients with endometriosis suffers from symptoms like painful periods (dysmenorrhea), non-menstrual chronic pelvic pain, pain due to intercourse (dyspareunia), and infertility.[2] There is little curative medical care. Surgical treatments often result in high rates of recurrence and loss of ovarian reserve, resulting in loss of fecundity.[1-3] The quality of life (QoL) in patients with this disease thus is significantly compromised, and it deteriorates even further due to the loss of productive time along with oft-present comorbidities, and resultant high healthcare spending.[2,4] Above all, the absence of robust diagnostic markers often results in delay of its early diagnosis and medical intervention.[5] Endometriosis appears to be a disorder with a variegated pathophysiological basis and disease manifestation. Endometriosis may present as superficial peritoneal endometriosis, ovarian endometriosis (or endometrioma), and deep infiltrating endometriosis (or rectovaginal nodules) with discernible histological differences.[6] Due to the inherent heterogeneity in phenotypes of endometriosis, the disease poses a serious challenge against attempts to improvise any simple operative approaches to the disease. Given the burden of individual stress, socio-economic strain, and clinician's anxiety linked to the disease, special attention for the development of non-invasive and minimally invasive but reliable diagnosis and classification of endometriosis appears a necessity. This would allow for the restriction of disease progression by its early detection and strategising towards its early treatment and management, and consequent avoidance of pain, stress, and invasive surgery. The aim of this article is to address the state of current knowledge regarding various diagnostic options as well as classification and staging systems available for endometriosis.

DIAGNOSTIC OPTIONS

The workup for diagnosis of endometriosis in a patient typically includes history noting and physical examination followed by clinical and imaging investigations, and laparoscopic examinations. History and physical examinations yield non-specific, but occasionally useful, information. Familial tendency and history of pain and infertility, palpable tender nodular masses on pelvic examination, and elevated cancer antigen-125, along with infertility and/or chronic pelvic pain provide important but generally non-specific cues. Among imaging techniques, ultrasound and MRI bear some diagnostic value, though those are not sufficiently specific and sensitive to different types of endometriosis and non-endometriotic lesions.[7-8] According to a Cochrane Database systematic review of imaging modalities for the non-invasive diagnosis of endometriosis, none of the imaging modalities were able to detect overall endometriosis with sufficient accuracy.[9]

Despite the fact that imaging may give useful indications, visual inspection of pelvic and extra-pelvic lesions at laparoscopy with histological confirmation remains the most reliable procedure towards the detection of endometriosis and its classification. Symptomatic individuals having likelihood of endometriosis are recommended to undertake laparoscopic examination. One out of 4 women who undergo a laparoscopic procedure due to symptoms of suspected endometriosis does not show endometriosis.[10] For a myriad of phenotypical issues, surgeons often face a diagnostic dilemma while inferring their observations.[8] In addition, laparoscopic examination is often hindered by the presence of dense pelvic adhesions.[8]

Histological confirmation in terms of any of the two features, namely endometrial glands, endometrial stroma, and hemosiderin-laden macrophages is the prerequisite of definitive confirmation of disease after visualisation of lesions. Two out of three patients with lesions considered to be endometriosis on laparoscopic examination were not histologically confirmed.[8,10] Additionally, false-negative results are often reported in cases of atypical lesions with histologically confirmed endometriosis.[10] Even with carefully conducted biopsy procedures by skilled surgeons and properly sampled specimens sent for pathologic examination, one-quarter of biopsy samples do not turn out to be endometriosis.[10,11] Nevertheless, laparoscopy is considered to be the standard modality for

the diagnosis of endometriosis.[7] Furthermore, laparoscopy can be applied for the treatments in which endometriomas may be cauterised or removed and adhesions can be lysed.[7] Laparoscopic surgery is, however, associated with an increased risk of intraoperative injury to bowel, bladder, ureter, and blood vessels.[10,11] Table 1 summarises some of the cardinal advantages and disadvantages of the above-mentioned different diagnostic methods.[7,10]

Table 1: Different diagnostic tools and their advantages and limitations.

Diagnostic tool	Advantage	Limitation
TVUS	• Minimally invasive. Accessible, inexpensive, fast, and safe. • Allows real-time assessment of pain and organ mobility. Particularly helpful for ovarian and bladder endometriosis. • Useful in planning and ENZIAN and FOATI scoring.	• Highly operator-dependent. • Limited to the focal length of the probe. • Non-specific to differential diagnosis with other ovarian lesions. • Ovarian cysts, subserosal leiomyomas, and acute retroflexion of the uterus, and also severe pelvic adhesions and other distortions of the pelvic anatomy may limit target visualisation.
REUS	• Minimally invasive. • Useful for patients with suspected DIE. • Provides a reliable method to evaluate intestinal wall infiltration. • Useful in ENZIAN and FOATI scoring.	• Highly operator-dependent. • Provides restricted view field. • Not useful for assessing ovarian, peritoneal, or anterior compartments.
MRI	• Non-invasive. • Excellent soft-tissue contrast with multiplanar capabilities and full panoramic and simultaneous assessment of both anterior and posterior compartments of pelvic structures. • Particularly useful in diagnosis of extensive pelvic adhesions and deep infiltration. • Useful in ENZIAN and FOATI scoring.	• Highly specialised, expensive, lengthy procedures. • Limited use in patients with pacemakers or cochlear implants, and with claustrophobia and/or morbid obesity. • Real-time evaluation is rare. • Bowel peristalsis may limit evaluation of intestinal DIE. Stool and gas may limit DIE visualisation.
Laparoscopy	• Highly standardised reference method. • Diagnostic as well as therapeutic.	• Invasive. • Requires skilled surgeons. Possibility of post-surgery issues of organ damage. • Low confidence in atypical cases, DIE, and in cases of adhesions. Digital images and visual inspection may yield different interpretations.

DIE: Deep infiltrating endometriosis; FOATI: foci–ovarian endometrioma– adhesion–tubal endometriosis–inflammation; REUS: rectal endoscopic ultrasound; TVUS: transvaginal ultrasound.

Adapted from Espada et al.[7] and Taylor et al.[10]

Given the insufficiency of available diagnostic tools, it is imperative that a biomarker-based approach may be devised to aid reliable diagnosis and classification of endometriosis. Typically, a biomarker is a characteristic that may be any substance, structure, or process in the body or its products and can be objectively measured and evaluated as an indicator of biological processes, normal or pathogenic, or pharmacologic responses to a therapeutic

intervention. The measurable entity may be functional and physiological, biochemical at the cellular level, or a molecular interaction.[12] The discovery of biomarkers with cues for diagnostic application potentially arises from a 'hypothesis driven' approach that screens a single molecule or a cohort of molecules involved in cardinal endometriosis-associated processes (e.g., neovascularisation, inflammation, cell survival, cell adhesion, cell proliferation and migration, and pain modulation).[13] On the other hand, a 'screening -omics approach' employs a relatively 'hypothesis neutral' paradigm to investigate and analyse multiple parameters (e.g., mRNAs, non-coding RNAs [ncRNAs], proteins, peptides, lipids, and classes) that are considered to be associated with the development and pathogenesis of endometriosis. Of all possible peripherally obtainable biological samples, urine, blood, and endometrium appear to be the obvious choices. In the following section, an account of state-of-the-art, clinically useful biomarkers as putative diagnostic options for endometriosis yielded from both 'hypothesis driven' and 'hypothesis neutral' approaches are presented. Table 2 provides a list of potential peripheral biomarkers of endometriosis.[13-28]

Table 2: Potential peripheral biomarkers for endometriosis.[13-28]

Target material	Name of the molecule(s)
Urine	• Cytokeratin-19 (CK19) • Histone-4 • Soluble *fms*-like tyrosine kinase (sFlt)-1 • Vitamin D binding protein
Blood	• α-1-B glycoprotein • Brain-derived neurotrophic factor • Cancer antigen-125 • Chemokine ligands (CCLs)-2, -5* • Chemokine ligand-8 • Glycodelin A • Haptoglobin • Hepatocyte growth factor • IL-1, -6, -8 • Matrix metalloproteinases-2, -3, -9 • Monocyte chemoattractant protein-1 • TNF-α • miRNA-28-5p, miR-29a-3p, 125b-5p
Endometrium	• Annexin-A2, -V • Erythroblastic leukaemia viral oncogene homologue receptors-1, -2 • Heat shock protein-90 • Platelet-derived growth factor receptor • miRNA-29C, miR-100, miR-200a, miR-200b

*Also known at RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted).

Markers may be specific to type and stage of the disease, and more correlated to specific symptom(s) of the disease (e.g., pain, infertility). None of these markers are fool-proof. A cohort of markers may present better specificity and sensitivity than any one of the biomarkers singularly.

Urinary Biomarkers

In a Cochrane Database systematic review, an attempt was made to assess the diagnostic performances of non-neural enolase (enolase-1), vitamin D binding protein, urinary peptide profiling, and cytokeratin-19.[14] The review reportedly failed to identify any significant differences in individuals with endometriosis from a disease-free control group.[14] In a comprehensive review analysing reported studies on the differential expression of urinary proteins as biomarkers of endometriosis, Gueye et al. drew a similar conclusion.[15] The results of a proteomic study indicated elevated histone 4 as a potential biomarker.[16] Also, a combination of four urinary proteins, namely histone 4, ADP-ribosylation factor 3, ribophorin 1, and myosin heavy chain 10 reflects significant promise of diagnostic value.[16] A secondary observation of the study that the high mobility group box 1, cluster of differentiation 40, and lymphotoxin β receptor signalling pathways were activated in endometriosis appears interesting.

Since these signalling pathways are integral to the inflammation process, the notion that chronic inflammation might take part in the development of endometriosis is being corroborated by this observation.[29,30] Thus, the combined urinary proteins may have significant promise for yielding cues for diagnostic and therapeutic options, but this requires further robust validation.

Circulatory Biomarkers

The results obtained from a multi-centre study have indicated CA-125 ≥30 unit/mL in peripheral circulation can act as a rule-in test for early diagnosis of endometriosis amongst women presenting with symptoms of pain and/or subfertility.[17] A multiplex profiling study of cytokines and angiogenic growth factors in plasma samples of patients with endometriosis and healthy controls revealed a potential panel of 14 cytokines (chemokine [C-C motif] ligand 2 [CCL2], CCL17, CCL21, CXCL5, CXCL11, CD14, carcinoembryonic antigen-related cell adhesion molecule 1 [CEACAM-1], erythroblastic leukaemia viral oncogene homologue 3 [ERBB3], IL-7, Lipocalin-2, neuronal cell adhesion molecule [NrCAM], receptor for advanced glycation end products [RAGE], TGF-β, and TNF-β) as a biomarker cohort with significance, specificity, and sensitivity to endometriosis disease samples.[18] It is however noteworthy that nine cytokines (shown above) revealed only marginal ($p<0.05$) differences in patients with endometriosis as compared to healthy controls and that a few (e.g., CCL21, IL-7, TGF-β) of those cytokines were seen to be differentially expressed in other inflammatory gynaecological disorders such as polycystic ovary syndrome, ovarian cysts, and pelvic adhesions.[18] Further studies on larger sample sizes with confirmed disease phenotypes are necessary to reach a point of useful clinical diagnostic option. It is notable in this regard that a Cochrane Database systematic review of 70 studies evaluating 47 blood biomarkers (angiogenesis factors, growth factors, apoptosis markers, cell adhesion molecules, high-throughput markers, hormonal markers, immune system markers, inflammatory markers, oxidative stress markers, microRNAs, tumour markers, and other proteins) with meta-analyses performed for four markers (anti-endometrial antibodies, IL-6, CA-19.9, and CA-125) failed to differentiate people with endometriosis from disease-free controls.[19]

With pain being a common symptom of endometriosis, several studies were performed to examine whether neurotrophic molecules detected in blood can be used as diagnostic markers. Brain-derived neurotrophic factor (BDNF) in circulation as a putative marker was reportedly able to differentiate cases between Stage I and Stage II endometriosis.[20] Although a higher serum level of mature BDNF was detected in those with self-reported pain with Stages I–II endometriosis prior to surgery, independent of menstrual cycle phase and irrespective of lesion type, the difference based on receiver operating characteristic curve analysis was not predictive for the disease.[21] In people with ovarian endometriosis and infertility with or without pain, BDNF levels in serum and peritoneal fluid were significantly higher in patients with pain but showed no association with the disease stages or menstrual cycle phases, however, correlated with BDNF mRNA and protein expression levels, and tyrosine receptor kinase B protein (receptor for BDNF and neurotrophin-3, -4 ligands) expressions in ectopic lesions in the presence of endometriosis pain.[22]

A large number of ncRNAs including microRNAs (miRNAs), long non-coding RNAs, and closed

long non-coding circular RNAs are involved in tissue-specific regulation of gene expressions at the transcriptional, post-transcriptional, and translational levels.[29] Thus, specific species of ncRNAs in peripheral biological samples, e.g., plasma, serum, saliva, and urine with high stability and pathophysiological relevance, bear potential biomarker value for various complex diseases, for example, endometriosis.[23-26,32] However, no circulating ncRNA has as yet been identified that could on their own comprise a reproducible, non-invasive diagnostic test for endometriosis. The observed lack of concordance between the reported studies could include geographic and ethnic differences in the expression of ncRNA repertoire, differences in sample handling, ncRNA extraction, normalisation, assay platforms, methods of statistical analysis, and the absence of a harmonised approach to tissue collection, storage, and of specimen characterisation on the basis of disease severity, disease phenotype, menstrual history, and fertility.[33-38]

Endometrial Biomarkers

Eutopic endometrium from patients with endometriosis differs from that of those without endometriosis.[13,27] It is commonly believed that endometrial biopsies collected using minimally invasive techniques with the aid of Pipelle or Karmen devices can be employed in the diagnosis of endometriosis. However, the dynamic nature of the cellular and molecular biology of endometrium, and additional complicating facets of the phenotypic and ethnic heterogeneity of endometriosis collectively pose challenges in the development of biomarker discovery for this disease.[28,39] In fact, a close survey of the literature reveals multiple caveats that would require close attention in future studies aimed at developing eutopic endometrium-based diagnostic targets for endometriosis. In the following section, the authors highlighted a few important issues in this regard.

- The menstrual cycle phase of tissue collection is a strong variable since the ratios of various endocrine factors differentially influence the cellular expressions of biomolecules in endometrium under disease compared to normal conditions.[13,35,40]
- Potential biochemical differences in lesion subtypes of peritoneal, ovarian, and deep infiltrating endometriosis are reflected in studies comparing eutopic endometria to control tissues.[13,41,42]
- The fertility status of individual patients may influence endometrial expressions.[37,43] Endometrial expressions may vary depending on the severity stages of disease in patients with positive fertility compared with infertile people with endometriosis.[44,45]
- The choice of endometriosis-free controls is an important issue since the presence of fibroids, adenomyosis, and/or pelvic organ prolapse may differentially affect the endometrial behaviour compared to that with no abnormality.[46] Furthermore, the choice of endometriosis-free control with pain and without pain is likely to display distinctions in the molecular expressions.[42,47] A high prevalence (approximately 45%) of asymptomatic cases (no pain or other symptoms) of endometriosis in individuals may cast significant skew in the control data.[48]
- The heterogeneity of tissue components that include inflammatory cells, stromal cells, epithelial cells, endothelial cells in uterine wall components, surrounding peritoneal tissue in different biopsy specimens *per se* may affect tissue expressional repertoire of tissue.[34,38,49]

Briefly, it appears that many peripheral biomarkers tentatively show promise in the diagnosis of endometriosis, but not a single biomarker or panel of biomarkers appears to be clinically fool-proof. It also appears that panels of markers rather than specific candidate markers may allow increased sensitivity and specificity for early diagnosis. Thus, after a decade of the reports based on systematic reviews of peripheral biomarkers of endometriosis by May et al., the present position remains similar: further research is warranted before any set of markers for endometriosis may be recommended for routine healthcare purposes.[50]

CLASSIFICATION AND STAGING OF ENDOMETRIOSIS

A reproducible classification system for a complex disease like endometriosis bears an advantage towards describing the pathological correlates of disease with acceptable levels of accuracy and precision, and also towards strategising effective medical and surgical interventions of the disease and disease-associated signs and symptoms.

Additionally, a simple and user-friendly classification protocol would render great help for communication between clinicians and other stakeholders, including patients.

Endometriosis can be classified according to its primary nidus (peritoneal, ovarian, rectovaginal etc.). Sampson classified the endometrioma into follicular, corpus luteal, stromal, and endometrial types depending on the presence of haemorrhagic cysts and adjoining adhesions.[51] On the basis of anatomical location, clinical findings, and histology, endometriosis may present as Sampson's syndrome (infertility and/or chronic dyspareunia with no deep pelvic local tenderness, induration or nodule formation, and histology showing superficial lesions of clear, red, black, or white lesions of endometrium-like glands and stroma), and Cullen's syndrome (tender palpable nodular or indurated lesion in the deep pelvis with histology of marked fibromuscular hyperplasia containing islands of endometrium-like glands).52 Endometriosis can also be classified as subtle, typical, cystic, deep, adenomyotic, and peritoneal pocket lesions estimated by their size.53 Ideally, a classification system should be able to identify disease morphology and severity with a high degree of accuracy and precision, and correlate the severity with the reported signs and symptoms of the disease (e.g., pain and subfertility).54 Accordingly, several attempts have been made to chronicle this evolving chronic disease in order to assess the stages and nature of lesions in association with pain scores and infertility.

In the following section, a summary of various classification systems available for endometriosis that include anatomical findings and disease staging based on imaging and laparoscopic investigations according to revised American Society for Reproductive Medicine (rASRM) and ENZIAN scores, and combinatorial approaches like Endometriosis Fertility Index (EFI) scoring and the FOATI systems is presented. Figure 1 provides the basic templates of the revised ASRM and ENZIAN protocols.

Revised American Society for Reproductive Medicine Scoring System

The American Fertility Society (AFS) introduced a scoring system for endometriosis in 1985 and a revised scoring procedure of the ASRM in 1996.55 According to rASRM, endometriosis is classified as superficial and deep lesions and staged as minimal (Stage I; Score: 1–5), mild (Stage II; Score: 6–15), moderate (Stage III; Score: 16–40), and severe (Stage IV; Score: >40). Some of the cardinal features of the rASRM scoring system are shown in Figure 1A. In the rASRM stages, weightage to endometriosis-associated visual landmarks at laparoscopy is attributed using arbitrarily designated scoring scales. This may lead to scoring of the disease to the same stage despite inherent differences in the nature of lesions, the latter having obvious bearing on strategising individual patient's treatment.56 The failure rate of such a protocol could reportedly be as high as 50%, and it is around 20% at best.57 Nevertheless, the rASRM protocol is widely practised for its ease to administer, report, and communicate, and for its apparent objective mode of presentation.

ENZIAN Classification System

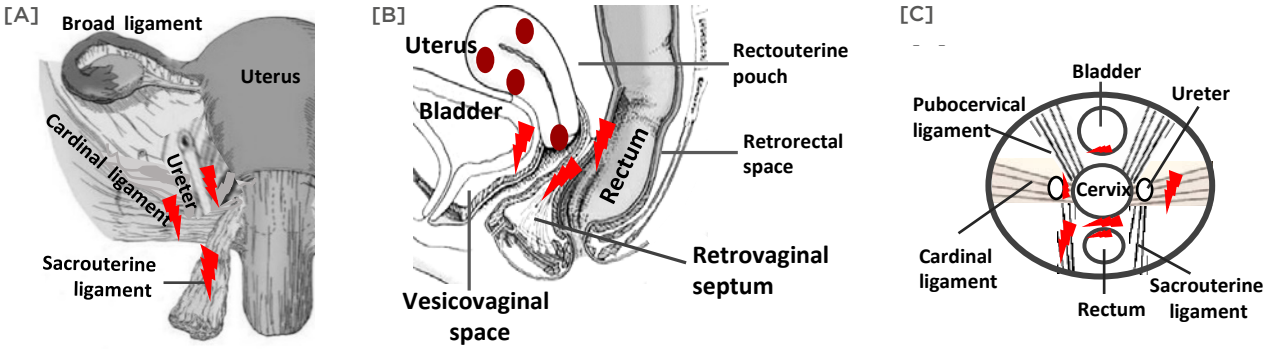
This classification system was introduced to supplement the rASRM system, especially taking into account deep infiltrating endometriosis and its involvement with other organs.58 ENZIAN classification was named after Hotel Enzian on Lake Weissensee in the Austrian Alps, where the 7th Conference of the Stiftung Endometriose Forschung (Foundation for Endometriosis Research), 25th–27th February 2011 developed this classification system. The original ENZIAN system was revised to reduce overlap with the rASRM system. In the revised ENZIAN classification system, the retroperitoneal structures are divided into three compartments: Compartment A consists of the rectovaginal septum and vagina; Compartment B consists of the uterosacral ligament and pelvic walls; and Compartment C consists of the sigmoid colon and rectum. The severity of the lesion is graded from its invasiveness (Grade 1: <1 cm; Grade 2: 1–3 cm; Grade 3: >3 cm). Deep endometriotic lesions in retroperitoneal distant locations (FA: adenomyosis; FB: involvement of the bladder; FU: intrinsic involvement of the ureter; FI: bowel disease caudal to the rectosigmoid junction; and FO: other locations, such as abdominal wall endometriosis) are also indicated in the system. A succinct coverage of the ENZIAN classification system is available in the 2020 recommendation of the Working group of the European Society

for Gynaecological Endoscopy (ESGE), the European Society of Human Reproduction and Embryology (ESHRE), and the World Endometriosis Society (WES).59 Some of the cardinal features of ENZIAN system are shown in Figure 1B.

A

Endometriosis	**<1 cm**	**1–3 cm**	**>3 cm**
Peritoneum superficial	1	2	4
Peritoneum deep	2	4	6
Right ovary superficial	1	2	4
Right ovary deep	4	16	20
Left ovary superficial	1	2	4
Left ovary deep	4	16	20
Posterior cul-de-sac obliteration			
Partial		4	
Complete*		40	
Adhesions†	**<1/3 enclosure**	**1/3–2/3 enclosure**	**>2/3 enclosure**
Right ovary filmy	1	2	4
Right ovary dense	4	8	16
Left ovary filmy	1	2	4
Left ovary dense	4	8	16
Right tube filmy	1	2	4
Right tube dense	4	8	16
Left tube filmy	1	2	4
Left tube dense	4	8	16
Assign point 16 in case of complete enclosure of fimbriated end of the fallopian tube. In case of patients with only adenexa, assigned points are to be doubled.			

(B)



(C)

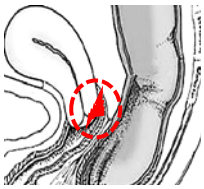
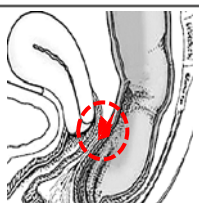
Compartment	A	B	C
Anatomical localisation	Rectovaginal space vagina	Sacrouterine and cardinal ligaments External ureter compression	Sigmoid colon Rectum
			

Figure 1:

A) Revised American Society for Reproduction Medicine (rASRM) scoring system for staging of endometriosis.[55] Determination of the stages of endometriosis based on examination of the pelvis at laparoscopy in a clockwise or counter-clockwise manner to note the number, size, and location of endometrial implants, plaques, endometriomas, and adhesions. The surface of the uterus is considered as peritoneum. Adhesions and lesions in the peritoneum, ovary, fallopian tubes, uterus, and cul-de-sac are scored as shown. Superficial peritoneal implants are shown as red, red-pink, flame-like, vesicular blobs. Clear vesicles, white opacifications, or haemosiderin deposits seen as black, blue deposits or yellow-brown deposits are also detected and scored. Adhesions are seen as filmy or dense, covering the ovary and tubes extending to the cul-de-sac, as shown in the different stages of endometriosis.
B) ENZIAN scoring system for deep infiltrating endometriosis.[59] The ENZIAN scoring provides a scoring of deep infiltrating endometriosis in retroperitoneal structures based on laparoscopic identification and the use of imaging (transvaginal ultrasonography, rectal endoscopic ultrasonography, MRI) techniques. The ENZIAN scores include lesions in the cul-de-sac, vagina, cervico-uterine ligaments, bladder, ureter, bowel, and uterus. The major anatomical sites of endometriotic lesions are sacrouterine ligament, cardinal ligament, and ureter [A]. Adenomyosis lesion sites in the uterus with the presence of heterotopic endometrial glands and stroma in the myometrium and reactive fibrosis of the surrounding smooth muscle cells of the myometrium, which often co-exists with endometriosis. Endometriotic lesion sites present in retrovaginal septum, bladder, vesicovaginal space, and retrorectal space [B] are shown. A schematic presentation of lesion sites detected within the pelvic compartment as shown in [A] and [B], excluding adenomyotic lesions, is presented in [C].

C) ENZIAN scoring system showing the levels (1–3) of deep endometriosis lesions that may be present in compartments like the rectovaginal space and vagina (A1–A3), sacrouterine, and cardinal ligaments to cause compression of external ureter wall (B1–B3) and the rectum (C1–C3). Other major lesion sites include adenomyosis (FA), lesions on the bladder (FB) and the ureter (FU). Endometriotic lesions may also be detected in extragenital sites such as the intestine, lung, and diaphragm, and in inguinal regions.

*Complete closure of the cul-de-sac by dense adhesions extending from the ovary and tube is scored as 40. The aggregation of points as shown indicates the endometriosis disease stages as minimal (I), mild (II), moderate (III), or severe (IV).

† Complete closure of the fimbriated end of the tube by adhesions is scored as 16.

Adapted from American Society for Reproductive Medicine 55 and Working Group of ESGE, ESHRE, and WES, Keckstein J et al.[59]

ENZIAN classification can be determined by imaging modalities and used for surgical planning, and it provides detailed descriptions of compartment-wise severity of lesion in the retroperitoneal structures, reportedly associated and correlated with the presence and severity of different symptoms (e.g., pain).[60,61] The ENZIAN classification system has as yet received only moderate reception, primarily due to its not being a user-friendly protocol and for not having an easy communication gait; the system is both complex and employs complicated terminologies. Also, there are currently no sufficient evidence-based reports regarding the usefulness of the ENZIAN classification system in determining pre-operative prediction regarding surgical decision.

Combinatorial Approaches: Endometriosis Fertility Index and Foci–Ovarian Endometrioma– Adhesion–Tubal Endometriosis–Inflammation–Adenomyosis– Recto-Vaginal Space System

Infertility is one major issue affecting the QoL of a large percentage of patients with endometriosis. The EFI provides a classification system on the basis of scores obtained from the assessment of surgical factors and historical factors, and projects to predict the clinical outcome of pregnancy in patients who are infertile[2,62] EFI is considered a valid clinical tool to predict fertility outcome for people following surgical staging of endometriosis and may be used for developing suitable treatment plans for infertile women with endometriosis.[2,62,63]

The Foci–Ovarian endometrioma– Adhesion–Tubal endometriosis–Inflammation–adenomyosis– Recto-Vaginal Space (FOATIaRVS) system of classification takes into consideration the histology of ectopic lesions and functional repercussions for tubal and ovarian functions along with the nature of inflammation. Collectively, it may help to identify the nature of medical and surgical treatments to be undertaken in patients who are infertile and have endometrioma; and the chances of malignant proliferation.[64,65]

To date, the authors have no template to classify 'atypical endometriosis', which is an intermediate precursor lesion linking typical endometriosis and clear cell/endometrioid tumours observed in 1–3 patients out of 100 endometriosis patients with endometrioma.[66] DIE, affecting 1–2% of individuals of reproductive age also bears the risk of developing malignancy.[67] Development of a classification system for assessment of endometriosis as a pre-malignant field defect which can be used for pre-emptive monitoring and management of the disease in a high-risk vulnerable population is seriously warranted.[68]

PRACTICAL PERSPECTIVE AND RECOMMENDATIONS

There is no fool-proof diagnostic option and classification or staging system for endometriosis disease. The core problem exists in the general failure of current systems to reflect a reproducible correlation with symptoms: infertility and pain, especially with the differential nature and severity of pain associated with endometriotic lesions in different compartments. Central and peripheral neural sensitisation and inflammation is causally associated with the pain caused from endometriosis, independent of anatomical distortion.

Conventional approaches to classifying endometriosis-associated pain based on disease, duration, and anatomy are grossly inadequate. Additionally, available diagnostic measures and classification systems fail to predict responses to medical and surgical interventions, disease recurrence, risks for associated disorders including malignancy, QoL measures, and other endpoints important to patients and healthcare providers for guiding appropriate therapeutic options and prognosis. In the given situation of a dearth of reliable diagnostic and classification systems, the WES recommends that clinicians adopt a 'toolbox approach' using all available diagnostic and classification systems, as appropriate, to maximise the information available to healthcare providers and patients.

It appears that the development of collaborative research study networks to harmonise the protocols for patient information, biological sample collection and their storage, and methodological and analytical tools, as well as applying those protocols to a wider patient base of diverse ethnicity and population is needed. This effort would pave the way to discovering reliable leads for future diagnostic options and a

classification-staging system helpful for an early diagnosis of the disease and its management. This, in turn, would give to the patients with this debilitating disease a fair chance to lead lives free of disease-associated stress.

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Conservative Management After a Single Fetal Death in a Dichorionic Diamniotic Twin Pregnancy and Fetus Papyraceus: A Case Report

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Abstract

Multifetal pregnancies are estimated to represent 3.2% of all pregnancies (80% are dichorionic and 20% monochorionic) and are associated with a higher risk of perinatal morbidity and mortality relative to single pregnancies. The authors report a successful case of conservative management of a dichorionic diamniotic twin pregnancy after a single fetal death in the second trimester of pregnancy. The diagnosis was made in the 22nd week of pregnancy and the pregnancy was followed up until delivery in the 39th week. A healthy (2.855 kg) female infant was born and a dead fetus, approximately 20 cm in length and connected by the rudimentary umbilical cord to the small calcified placenta, was seen. The occurrence of a single fetal death is a relatively common event, which has implications for maternal and fetal outcomes. This diagnosis is relevant due to its potential effect on the survival of the other fetus and on possible maternal complications. In order to avoid complications and achieve the optimal maternal and neonatal outcomes, conservative prenatal follow-up should focus on careful monitoring and serial assessment of both fetal and maternal wellbeing. In gestational losses where the fetus is retained intrauterine for at least 10 weeks, there is the possibility of finding fetus papyraceus at the time of delivery. This is a rare event that results from incomplete reabsorption of the dead fetus, which is then compressed between the membranes and the uterine wall.

INTRODUCTION

Multifetal pregnancies are estimated to represent 3.2% of all pregnancies (80% are dichorionic and 20% monochorionic)[1] and are associated with a higher risk of perinatal morbidity and mortality when compared to single pregnancies.[2-4] The occurrence of a single fetal death is a relatively common event, occurring in approximately 6% of cases,[2,4-6] and has several implications for maternal and fetal outcomes, such as maternal disseminated intravascular coagulation, hypertensive disorders of pregnancy, and structural abnormalities in the surviving fetus

(e.g., cerebral alterations, bilateral renal cortical necrosis, gastrointestinal tract atresia, and aplasia cutis).[7] The extrinsic compression of the dead fetus by the remaining fetus can result in the appearance of an entity known as fetus papyraceous, a rare event that occurs in 0.018–0.02% of multifetal pregnancies.[8,9]

Herein, the authors report a successful case of conservative management of a dichorionic diamniotic twin pregnancy after a single fetal death in the second trimester of pregnancy.

CASE REPORT

A 36-year-old patient (gravida 2, para 1; one previous caesarean section) was referred for follow-up at the high-risk prenatal care unit of a tertiary referral hospital due to a dichorionic diamniotic twin pregnancy at 11 weeks of pregnancy (calculated by an obstetric ultrasound performed at the 8th week). The patient was known to have epilepsy, controlled using phenobarbital (100 mg daily), but no other comorbidities were recorded. At the 13th week of pregnancy, another obstetric ultrasound was performed, showing two gestational sacs of usual aspect, containing, in each one, an embryo with a visible heartbeat and nasal bone. In addition, nuchal translucency screening tests were in the appropriate percentiles for gestational age (Figures 1 and 2). At the 16th week of pregnancy, the patient was clinically examined and both twins' hearts were auscultated. No abnormalities were found on the clinical examinations.

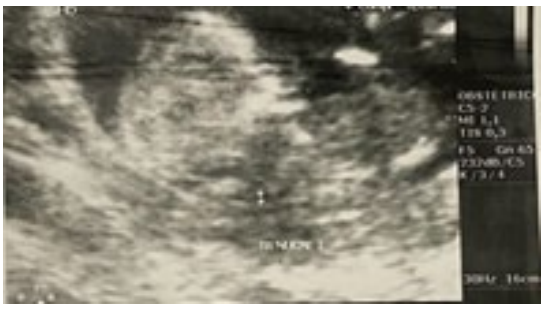

Figure 1: Ultrasound showing first twin with normal nuchal translucency screening and presence of nasal bone.

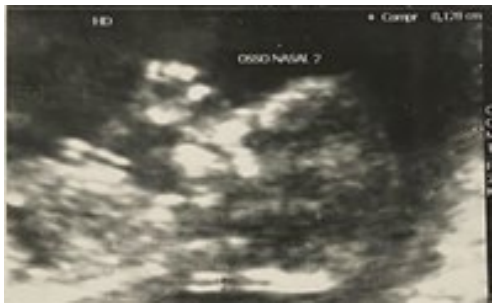

Figure 2: Ultrasound showing second twin with normal nuchal translucency screening and presence of nasal bone.

At the 22nd week of pregnancy, a morphology ultrasound was performed and the death of one of the fetuses was diagnosed. The live fetus showed good vitality and no morphological changes were detected. The dead fetus was not described.

The pregnancy follow-up comprised a total of 12 prenatal visits and all tests recommended were performed, including three more ultrasound examinations, which were performed at 23–24 weeks, 31–32 weeks, and 37–38 weeks. These consistently demonstrated a fetus with adequate growth and good vitality. There were

no descriptions regarding the dead fetus in the ultrasounds.

At 39 weeks of gestational age, the patient was admitted to the maternity ward for caesarean section on request (according to the maternity protocol). Gestational hypertension was diagnosed on admission. The patient underwent an uneventful lower segment caesarean section. A healthy (2.855 kg) female infant was born, whose Apgar scores were 8 and 9 at the first and fifth minute of life, respectively. After extraction of the placenta, a dead fetus, approximately 20 cm in length and connected by the rudimentary umbilical cord to the small calcified placenta, was seen (Figure 3). The set of placentas and the mummified fetus were sent for anatomo-pathological examination. The mother and the newborn had no complications during hospitalisation and were discharged 72 hours after delivery.

The anatomo-pathological report showed a succenturiate placenta (an accessory lobe) and a marginally inserted umbilical cord containing three vessels. No other relevant histopathological lesions were observed.

Adhered to the membranes was an involuted placental disc of 15.0x12.0x0.3 cm with extensive areas of infarction/hyalinisation and calcification. In the same flask, there were a fetus papyraceus showing maceration Grade III, whose sex could not be morphologically determined, with an anthropometric gestational age of 13 weeks.

DISCUSSION

This report shows that conservative management can be successful for cases of single fetal death in a twin pregnancy during the second trimester. The prognosis of pregnancy after the death of one of the twins will depend primarily on the gestational age at the time of fetal death and chorionicity, regardless of amnionicity. If the loss occurs within the first trimester, the death of one of the fetuses does not appear to be associated with deleterious effects on the development of the survivor, especially in dichorionic diamniotic pregnancies. In this instance, patients may be asymptomatic or present with abdominal pain and mild genital bleeding.[2,8,9] However, single fetal death after 14 weeks, and especially after the 20th week of pregnancy, is associated with adverse effects on the surviving fetus, with a higher risk of prematurity (spontaneous or iatrogenic), restricted intrauterine growth, neurological morbidity for the surviving fetus, pre-eclampsia, haemorrhage, and sepsis.[2,4,5,8,10]

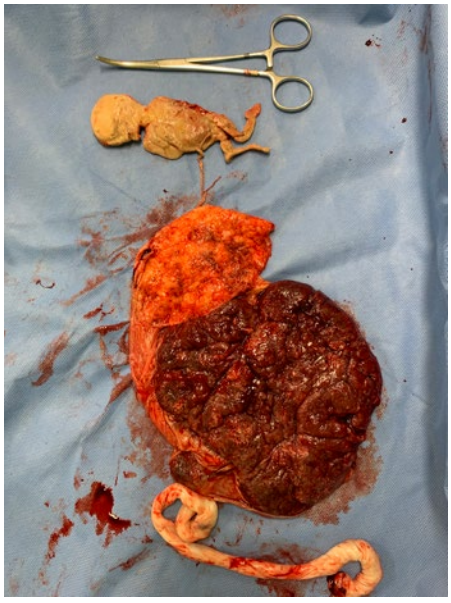

Figure 3: Macroscopically unchanged placenta adhered to calcified placental disc, which is connected by a rudimentary umbilical cord to a mummified fetus.

The prognosis of the surviving fetus is worse in monochorionic pregnancies, regardless of amnionicity, due to mechanisms that are not yet well understood. However, it is most probably related to the presence of important vascular anastomoses that allow thrombotic substances released by the dead fetus to reach the circulation of the surviving fetus, causing hypotension, hypoperfusion, hypoxia, acidosis, exsanguination, severe anaemia, and generalised ischaemic injuries (particularly in the central nervous system of the surviving twin).[9,11,12]

In monochorionic twins, the risk of prematurity (most relevant between 28–33 weeks of pregnancy), neuropsychomotor disorders, postnatal cranial imaging abnormalities, and death of the surviving twin after single fetal death were estimated at 68%, 26%, 34%, and 15%, respectively, while in dichorionic twins the rates were estimated at 54%, 2%, 16%, and 3%, respectively.[10,12]

Currently, there is no consensus regarding the follow-up or definition of the ideal gestational age for interruption of pregnancy in the event of the death of one of the fetuses in twin pregnancies. However, fetal death in the first trimester does not appear to be associated with adverse outcomes, a risk that increases from the second or third trimester.[11-13] In these scenarios, the conservative approach is advocated above all else when gestational loss of one of the fetuses occurs at a non-viable gestational age or is associated with extreme prematurity.[13] In the case of dichorionic pregnancies, pregnancy must be carried out for at least 38 weeks, whenever both maternal and fetal wellbeing are assured,[6,11,13] unless there is some other obstetric indication for termination of pregnancy. In the case of monochorionic pregnancies, conservative management is an option, especially prior to 34 weeks, because of the greater neonatal risks associated with prematurity. Antenatal corticosteroid therapy should be considered.[13]

In conservative management, follow-up should prioritise surveillance of fetal wellbeing through serial ultrasounds to monitor fetal growth and amniotic fluid volume. Doppler ultrasonographic measurement of the peak systolic velocity in the middle cerebral artery is a good parameter for monitoring fetal anaemia. This is very useful when assessing wellbeing due to the subsequent risk of exsanguination of the surviving fetus.[13,14] Persistent absent or reversed end-diastolic flow in umbilical artery Doppler has been associated with severe fetal deterioration, while intermittent absent or reversed end-diastolic flow has been reported to be associated with unexpected fetal demise. Normal umbilical artery Doppler pulsatility index carries the best prognosis.[11,13,17]

For maternal monitoring, coagulation blood tests are recommended. Moreover, special attention should be paid to blood pressure and proteinuria levels. This is due to the higher risk of hypertensive disorders associated with twin pregnancies, especially those in which one of the fetuses dies.[13,15] Anti-Rho Ig should be administered to rhesus-negative patients.[13] The determination of the mode of delivery must be based on obstetric criteria.[11,13]

Fetus papyraceous results from incomplete absorption of a dead fetus, retained inside the uterus for at least 10 weeks, which undergoes fluid loss and mechanical compression between the membranes and the uterine wall. The cause of death is generally unknown but it is often associated with chromosomal abnormalities, placental abnormalities such as improper insertion of the umbilical cord, or, in the case of monochorionic diamniotic pregnancies, twin-to-twin transfusion syndrome.[8] The cause of death of one of the fetuses in this case report was unknown. However, it is important to discuss one of the relevant comorbidities of the patient: epilepsy, which was treated with phenobarbital (100 mg daily). There are data in the literature showing that exposure to anti-epileptic drugs, especially phenobarbital, may be associated with unfavourable pregnancy outcomes. In a study conducted by Wen et al.,[16] 14,982 women were exposed to folic acid antagonists (either sulfamethoxazole–trimethoprim or phenobarbital). Overall, maternal exposure was associated with a greater risk of restricted fetal growth and fetal death. A prospective case-control cohort study conducted by Waters et al.[17] also showed that women with epilepsy who were exposed to anti-epileptic drugs (carbamazepine, phenytoin, and phenobarbital) had a higher rate of fetal death and anomalies than the control population. In this instance, phenobarbital was associated with the highest relative risk for those outcomes. No data have been found in the literature concerning single fetal death in

dichorionic and diamniotic pregnancy related to phenobarbital exposure.

CONCLUSION

The diagnosis of a single fetal death in multifetal pregnancies is relevant due to its potential effect on the survival of the other fetus and on possible maternal complications. In order to avoid complications and achieve the optimal maternal and neonatal outcomes, conservative prenatal follow-up should focus on careful monitoring and serial assessment of both fetal and maternal wellbeing.

In gestational losses where the fetus is retained intrauterine for at least 10 weeks, there is the possibility of finding fetus papyraceus at the time of delivery. This is a rare event that results from incomplete reabsorption of the dead fetus, which is compressed between the membranes and the uterine wall.

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Physiotherapy in Urinary Dysfunction Post-Surgery for Endometriosis: Case Report

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Abstract

Endometriosis is characterised by the presence of endometrial tissue outside the uterine cavity that responds to oestrogen and stimulates local inflammatory processes, adhesions, pelvic pain, and infertility. The treatment of endometriosis includes the use of medications and videolaparoscopy for excision of adhesions or lesions. Some complications are associated with the videolaparoscopy, such as vascular, intestinal, urinary, neurological, and more rarely, vulvar oedema, which makes the rehabilitation difficult in the immediate postoperative period. In relation to the urinary dysfunction and to the vulvar oedema, physiotherapy has resources with demonstrated efficacy in the treatment of such complications after videolaparoscopy; they can rehabilitate the patient and improve their quality of life in a short time. In this study, the authors report the case of a patient treated by the Physical Therapy Service of the Santa Casa de Misericórdia Hospital of São Paulo, São Paulo, Brazil, with urinary retention and vulvar oedema after videolaparoscopy for endometriosis, which showed improvement in vulvar oedema with the application of physiotherapy.

BACKGROUND

Endometriosis is a chronic condition, characterised by the presence of endometrial tissue outside of the uterine cavity, such as the pelvic cavity, the rectum, the ovary, and the uterosacral ligament and in rare cases also in the diaphragm, pleura, and pericardium. Endometriosis responds to oestrogen hormone stimulation, which stimulates local inflammatory processes, accompanied by adhesions, fibrosis, neuronal infiltration, and anatomical distortion, which contribute to the presence of pelvic pain and infertility.[1-3]

Endometriosis affects 6–10% of females of reproductive age and is seen in up to 60% of those who present with pelvic pain complaint and in up to 50% of cases of infertility.[3] The diagnosis is based on the clinical history of the patient, signs and symptoms, physical examination, medical imaging techniques (e.g., MRI), and proven by histological examination of the materials collected during the laparoscopy.[4] Several theories attempt to explain the pathogenesis of the condition and can be divided into those that suggest that the implants originate from endometrial tissue, such as the retrograde menstruation theory, and those that propose a nonuterine origin, such as the theory of coelomic metaplasia.[5,6]

According to the American Society for Reproductive Medicine (ASRM), endometriosis can be classified into four stages (mild to severe), with different degrees of impairment and affected areas in each of them.[7] The treatment for endometriosis includes oral progestogen contraceptives, testosterone-derived agonists of gonadotropin releasing hormone, and nonsteroidal anti-inflammatory drugs.[8] Surgery plays an important role in the control of endometriosis and aims to completely ablate and/or excise the endometrial tissue and to correct anatomical changes caused by adhesions or lesions.[9] Laparoscopy is considered to be the gold standard test for the diagnosis of this pathology and resection of endometriosis through this technique is effective in pain control.[10] Vulvar oedema and urinary retention are some of the urinary complications related to videolaparoscopy.[9]

Postlaparoscopic vulvar oedema is a rare complication, but when presented it may affect the patient's urination. Unfortunately, there are still no guidelines for its management and the physiopathology associated with the procedure is unknown.[11] The studies of Guven et al.[11] and of Trout et al.[12] reported cases of patients who underwent laparoscopic surgery and presented with vulvar oedema in the postoperative period. Consequently, urination was impaired and relief catheterisation to decrease the oedema and rest were required. The authors observed that the decreasing of oedema was associated with an improvement in diuresis. It is important to mention that vulvar oedema can also have other causes, such as vulvar trauma as a result of uterine manipulation during hysterectomy and voiding dysfunction in the postoperative period of females with deep endometriosis undergoing surgery can be secondary to other causes (neurogenic dysfunctions of the pelvic floor).

CASE REPORT

Described here is the physiotherapeutic intervention in vulvar oedema and urinary retention presented in the postoperative period of videolaparoscopy for the treatment of endometriosis.

The case report followed the sequence of identification, main complaint, history of the current disease, and evolution. The research instruments used in this case were anamnesis, evaluation of the pelvic floor with visual inspection, and physiotherapeutic care (seven sessions).

A 45-year-old female of Caucasian background was diagnosed with endometriosis, on the 3rd postoperative day of right oophoroplasty with hysterectomy and bilateral salpingectomy and right pararectal tumour excision. Following removal of the bladder catheter, the patient developed urinary retention and urinary incontinence as a result of overflow; the catheter was inserted again and maintained. The patient was evaluated in bed by the Pelvic Physiotherapy Service of the Irmandade da Santa Casa de Misericórdia de São Paulo on the fifth postoperative day. In the inspection, vulvar oedema and altered sensitivity of the external region of the vulva were observed (Figure 1A). Sensitivity in the vulva region was assessed by the Semmes-Weinstein Monofilament (SWM) test. The patient was instructed by the

physiotherapy team to apply cryotherapy (ice pack) to the vulvar region for 20 minutes and perform stimulation in the inguinal lymph nodes (20 circles in the inguinal lymph node chain), twice a day.

The patient was discharged on the 5th postoperative day and returned to the physiotherapy outpatient clinic after 48 hours for the first session of physical therapy, already showing improvement of the oedema (Figure 1B).

During the first postdischarge session, functional electrical stimulation was performed in the supra pubic region and in the labia majora (Figure 2), with a pulse width of 500 microseconds, 50 Hz frequency, 2-second ramp rise, 5-second maintenance, 2-second ramp descent, with an application time of 10 minutes.[13,14] A device with two channels was used: adhesive electrodes (5 cm x 5 cm) were applied in the supra pubic region and adhesive electrodes (3 cm x 5 cm) in the labia majora; the application was simultaneous. The purpose of electrostimulation was to activate muscle contraction to perform a mechanism of pumping the accumulated liquids in the application regions.[13,14]

Transcutaneous electrical nerve stimulation current was also applied in the presacral region (S2 to S4) for neuromodulation, with a pulse width of 250 microseconds and a frequency of 8 Hz for 30 minutes, aiming to improve urinary retention. The patient was instructed to perform perineal exercises 3 times a day (the patient was in the supine position, with abducted hips, bent knees, and feet on the bed). The exercise consisted of contracting the pelvic floor for 5 seconds and relaxing it for 10 seconds, for 5 times. The patient was also instructed to maintain the application of cryotherapy to the vulvar region as well as the stimulation of the inguinal lymph nodes (20 circular movements).

During the second physiotherapy session, performed in the same week, the patient was no longer using a bladder catheter, the oedema had decreased, and they reported a sensation of incomplete emptying of the bladder (Figure 3). The conduct was maintained, and the patient was instructed to perform scheduled urination every 3 hours.

At the third session, the patient no longer presented with oedema, they reported a strong urinary stream, good urine flow, and postvoid residual; the conduct was maintained until the sixth session.

GENERAL CONSIDERATIONS

Operative laparoscopy has undergone technological advances in the last decade.[11] According to several studies, vulvar oedema is a complication that rarely occurs in the postoperative period of laparoscopy, which may explain the lack of studies on the subject.[11,12,15,16] The first description of this complication, related to laparoscopic surgery, was made by Trout and Kemmann in 1996.[12] The researchers reported a case of three patients undergoing laparoscopy for treatment of ovarian cyst, pelvic adhesions, and gamete intrafallopian transfer, who presented with vulvar oedema in a 24-hour postoperative period, as well as discomfort and inability to urinate. The patients were hospitalised and received traditional treatment with Foley catheter for urine drainage, local application of ice, and rest. Their condition improved between 1 and 3 days, but one patient developed a urinary tract infection. The researchers also reported that in their 3 years' of experience, they only observed three cases of vulvar oedema among approximately 900 laparoscopies, which confirms the low rate of occurrence of this complication. The studies of Pados et al.[15] and Guven et al.[11] reported vulvar oedema in laparoscopic cystectomy. In the report by Pados et al., the patient presented with discomfort and vulvar oedema 3 hours after the surgery. The treatment consisted of a Foley catheter, introduced to prevent urinary retention due to the worsening of their condition; application of ice to the vulva; rest for the patient; and bandaging of the oedema after application of topical steroid cream. The oedema improved after a few minutes and the catheter and the bandage were removed after 48 hours and the patient was discharged from hospital. In the report by Guven et al., the patient was readmitted 27 hours after the procedure with vulvar oedema and inability to urinate. In parallel to the study by Pados et al., the treatment was performed with application of ice to the vulva, Foley catheter, and rest, with improvement of the oedema in 30 hours and hospital discharge after 2 days, without complications. These results are similar to the case report presented here.

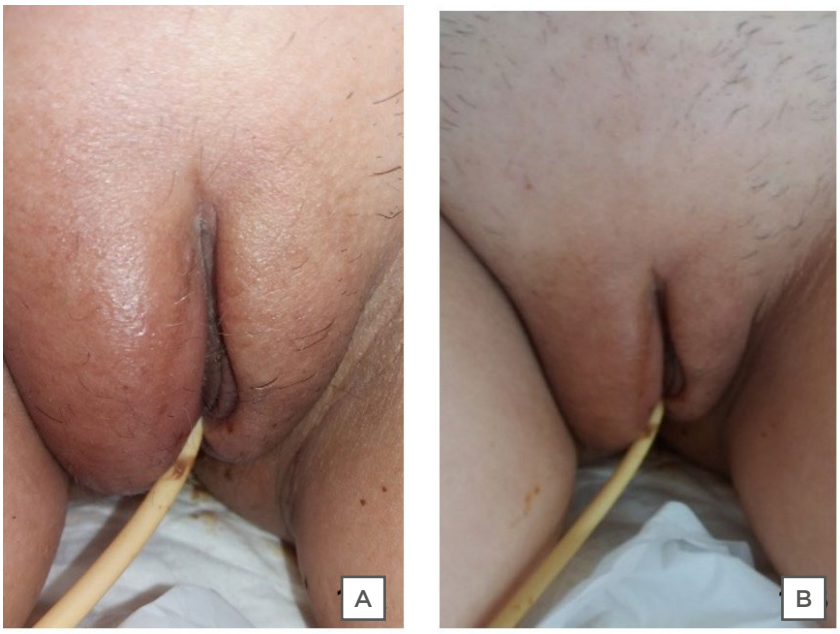

Figure 1: Postoperative vulvar oedema.

A) Vulvar oedema at the time of evaluation of physical therapy on the 5th postoperative day. **B)** Improvement of the oedema and of urination after 48 hours of hospital discharge.

Source: Pelvic Physiotherapy Outpatient Clinic of the ISCMSP (Irmandade da Santa Casa de Misericórdia de São Paulo).

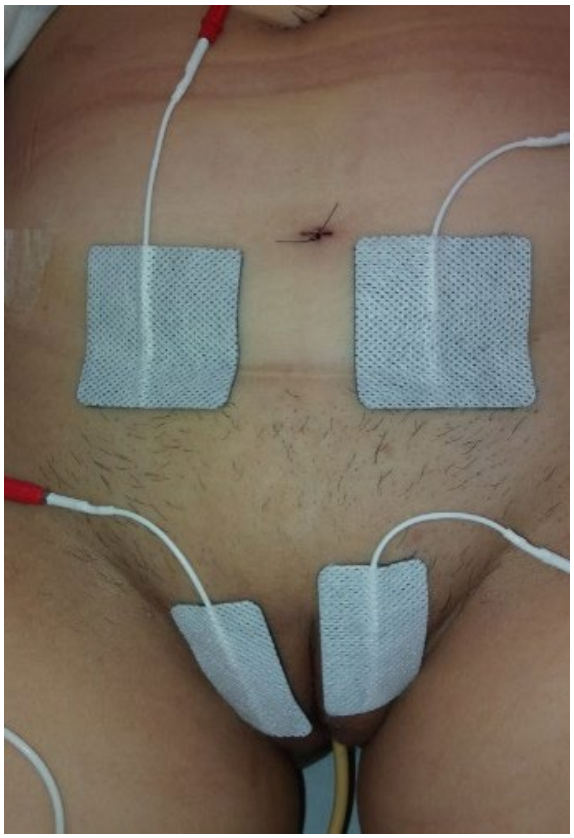

Figure 2: Surface electrostimulation.

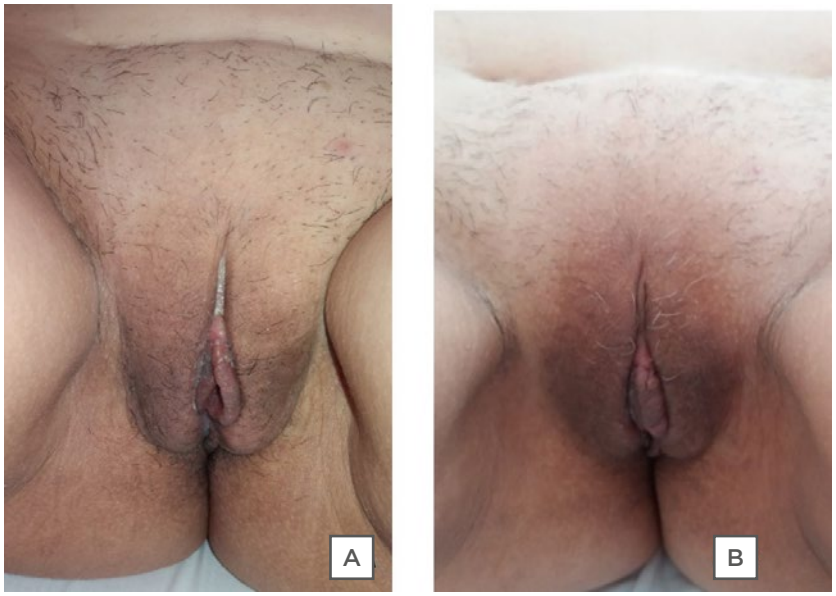

Figure 3: Decreased vulvar oedema during physiotherapy treatment.

A) During the second session of physiotherapy, the patient did not require the use of a bladder catheter and showed improvement of the oedema and urinary retention. **B)** During the seventh session, the patient had no complaints and no urinary retention; they were discharged from physiotherapy treatment.

It was possible to observe that the time of onset of vulvar oedema in the postoperative period and the time elapsed for its improvement varied in all the studies. A common complication was urinary retention, a consequence of vulvar oedema, which makes it impossible for the urine to be released as the oedema compresses the urethra. Its cause is complex and unpredictable, and its incidence rate associated with laparoscopy varies between 1.2% and 22.9%.[16] The pathophysiology of vulvar oedema is still unknown.[11] One of the hypotheses is the intraoperative or post-surgery migration of an intra-abdominal fluid from a suprapubic puncture area to the vulvar subcutaneous tissue.[15,16] The persistence of the canal of Nuck may also be one of the causes of fluid leakage from the peritoneal cavity to the vulvar region.[11,15,17,18] The canal of Nuck is a small wrapping of the parietal peritoneum, it is connected to the uterus by the round ligament through the inner inguinal ring in the inguinal canal and it communicates with the labia majora. It is a predelivery canal, which is closed at birth or disappears in the first year of life. When this communication remains open it may be the cause of the inguinal hernia and of the hydrocele of the canal of Nuck.[18,19] Reed and Robinson[18] related the use of 4% icodextrin in laparoscopic gynaecological surgery to vulvar oedema and urinary retention presented by one patient. Icodextrin is used laparoscopically at the time of surgery to prevent the formation of adhesions that can lead to complications such as infertility, chronic pelvic pain, and small intestine obstruction. The authors reported the case of a patient readmitted after laparoscopic salpingectomy using 4% of icodextrin to prevent adhesions. The patient complained of inability to urinate and presented significant vulvar oedema on physical examination. According to the authors, few complications are reported in the literature associated with the use of this substance, and the mechanism of vulvar oedema associated with it is unknown. However, they considered the patient's persistence of the canal of Nuck as a possible cause of vulvar oedema, since the substance is expected to remain in the peritoneal cavity until it is absorbed by the lymphatic system.

Regarding the management of vulvar oedema, it was observed that cryotherapy was a common therapeutic resource in the case reports discussed here, possibly because it presents results in the control and treatment of acute oedema with solid physiological and well-established bases in the literature, in addition the fact that it is a resource of low cost and easy application.[20,21] The intervention in the management of vulvar oedema

the authors applied was composed, in addition to cryotherapy, of electrostimulation, perineal exercises, and inguinal ganglion stimulation, which together presented excellent results. However, citations about the application of these resources in the other reports presented here were not found in author publications. The authors also did not comment if physical therapy was performed at some point during the treatment of the patients. Pinto e Silva et al.[22] reported cases of four patients with vulvar oedema due to different aetiologies: one case of cervical cancer, one case of bilateral adrenalectomy postoperative for pheochromocytoma, and two cases of pregnancy with pre-eclampsia. The physical therapy treatment consisted of manual lymphatic drainage in the vulva region; stimulating lymphatic flow towards the inguinal lymph nodes; multilayer compression therapy, with overlapping bandages throughout the genital area, arranged as an underwear, aiming at lymphatic reabsorption and stimulation of lymphatic transport; and skin care. The physiotherapists used their knowledge of applying compressive bandages to upper and lower limbs to adapt the technique to the genital area. The researchers observed that this intervention had faster results in the resolution of vulvar oedema (mean: 3.5 days) compared with other studies in which the oedema was not treated, or other types of intervention were used.

CONCLUSION

The physiotherapeutic performance presents useful knowledge and resources to assist in the treatment of vulvar oedema and urinary retention. These complications are rare, but they may occur in the postoperative period for the treatment of endometriosis. The importance of physical therapy practise in these complications should be emphasised. Although endometriosis has no cure, it can be treated and controlled through periodic follow-up.

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Diagnostic Criteria and Treatment Modalities of Ectopic Pregnancies: A Literature Review

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Abstract

Background: Ruptured ectopic or extrauterine pregnancy (EP) is responsible for 6% of maternal deaths in the first trimester. This review was designed to summarise the diagnostic criteria and treatment modalities of EPs.

Methods: Recent guidelines of the international societies of obstetrics and gynaecology, including the Royal College of Obstetricians and Gynaecologists (RCOG), the American College of Obstetricians and Gynecologists (ACOG), and the European Society of Human Reproduction and Embryology (ESHRE), were reviewed to summarise the diagnostic criteria and treatment modalities of EPs.

Results: A minimum β-human chorionic gonadotropin (β-hCG) rise of ≥35% in 48 hours was suggested to diagnose intrauterine pregnancy. A β-hCG rise <35% in 48 hours has 96.2% positive predictive value, 69.7% negative predictive value, and 80.2% overall accuracy in predicting EPs. The blob sign has >90% positive predictive value in diagnosing EPs in symptomatic females with positive β-hCG and no definite intrauterine gestational sac by transvaginal sonography. The interstitial ectopic pregnancy and cornual pregnancy are two separate entities of EPs. Interstitial line sign has 80% sensitivity and 98% specificity in diagnosing interstitial ectopic pregnancy. A meta-analysis reported 89% overall success rate for methotrexate in treatment of EPs; the multi-dose regimen was significantly more successful than the single-dose regimen.

Conclusion: Institutes and healthcare providers should follow clear guidelines and/or protocols for the management of EPs. Institutes should implement competency-directed training programmes to increase healthcare providers' skills to diagnose and treat EP variants using different modalities.

INTRODUCTION

Ectopic or extrauterine pregnancy (EP) is a first trimester pregnancy complication that occurs in 1.3–2.4% of all pregnancies.[1] Ruptured EP is responsible for 6% of maternal deaths in the first trimester.[1,2] The increased rates of assisted reproductive techniques (ARTs), tubal surgeries, and improved diagnostic tools are responsible for increased incidence of EPs.[1]

A minimum β-human chorionic gonadotropin (β-hCG) rise of ≥35% in 48 hours was suggested to diagnose intrauterine pregnancy (IUP). A β-hCG rise <35% in 48 hours has 96.2% positive predictive value (PPV), 69.7% negative predictive value, and 80.2% overall accuracy in predicting EPs.[3] The blob sign has >90% PPV in diagnosing EPs in symptomatic females with positive β-hCG and no definite intrauterine gestational sac (IUGS) by transvaginal sonography (TVS).[2]

The interstitial ectopic pregnancy (IEP) and cornual pregnancy are two separate entities of EPs. Interstitial line sign has 80% sensitivity and 98% specificity in diagnosing IEP.[4-9]

The American College of Obstetricians and Gynecologists (ACOG) reported spontaneous resolution of EPs in 88% of patients at initial β-hCG <200 mIU/mL following expectant managment.[10]

A meta-analysis reported an 89% overall success rate for methotrexate (MTX) in treatment of EPs; the multi-dose regimen was significantly more successful than the single-dose regimen but caused more side effects.[11] Despite the available diagnostic tools, delayed diagnosis and unreliable follow-up make ruptured EPs a daily practice in obstetrics and gynaecology. This literature review was designed to summarise the diagnostic criteria and treatment modalities of EPs.

DIAGNOSTIC CRITERIA AND TREATMENT MODALITIES OF ECTOPIC PREGNANCIES

EPs occur following implantation of the fertilised ovum outside the normal uterine cavity.

Classification of Extrauterine Pregnancies

- Tubal EPs. These constitute >90% of EPs. In 80% of cases, tubal EPs occur in the ampullary region of the fallopian tube (FT). It may occur in other parts of the FT, including isthmus, fimbria, or the interstitial portion.
- Non-tubal EPs. These constitute <10% of EPs and may be cervical, ovarian, intramural, or abdominal.
- Heterotopic pregnancies (HPs).
- Caesarean section (CS) scar pregnancies (CSSP).

Incidence of Extrauterine Pregnancies

- Tubal EPs occur in 1.3–2.4% of all pregnancies.[1] IEPs account for 4% of EPs.
- Non-tubal EPs:

1. Cervical EPs occur in 1:2,000–1:18,000 pregnancies. The incidence of ovarian EP is 2% and is the most common type of non-tubal EP.[12]
2. Intramural EP (pregnancies within the myometrium) account for 1% of EPs. Abdominal pregnancy (AP) accounts for 1.3% of EPs.[13]
3. HPs occur in 1:4,000–1:30,000 in the general population.
4. CSSP incidence rate increased following the increased caesarean section rates, and occurs in 1:1,800–1:2,216 pregnancies.[2]

Taran et al.[1] explained the rising incidence of EPs by the increased rates of ARTs, tubal surgeries, and improved diagnostic tools.[1] Perkins et al.[14] reported that the incidence of EPs following ARTs has decreased over time but the risk of EP increased following multiple embryo transfers during ARTs.[14] In addition, the ACOG reported the multiple embryo transfers and tubal factors of infertility as risk factors for EPs following ARTs.[10] Ruptured EP is responsible for 6% of maternal deaths in the first trimester.[1,2] A review of mortality in ART-associated EPs reported a mortality rate of 31.9 deaths per 100,000 pregnancies.[15]

AETIOLOGY OF EXTRAUTERINE PREGNANCIES

The FT is the site of oocyte fertilisation. The migration of the fertilised oocyte to the uterine cavity for implantation is mediated by the FT smooth muscles and cilia. FT inflammation and/or dysfunction are implicated in oocyte retention and subsequent EPs.[2]

Risk Factors for Tubal Extrauterine Pregnancies

Up to 50% of females diagnosed with EPs have no identifiable risk factors; however, several risk factors have been associated with EPs.[1] High-risk factors (odds ratio [OR]: >4.0) include prior tubal surgery, prior EP, sterilisation, and

intrauterine device (IUD) users.[1] Moderate risk factors (OR: >2.0) include infertility, current or prior pelvic inflammatory disease, smoking, and more than one sexual partner.[1] Mild risk factors (OR: <2.0) include age >40 years.[1] IUD use decreases the overall pregnancy rates and prevents intrauterine implantation, with subsequent higher incidence of EPs in females conceiving with an IUD in place compared to the general population.[11]

Risk Factors for Non-tubal Extrauterine Pregnancies

Previous dilation and curettage are risk factors of cervical EPs. Myometrial injury during curettage, myomectomy, or CS is a risk factor for intramural EPs. Pelvic inflammatory disease, ARTs, and endometriosis are risk factors for APs.[1]

Risk Factors for Caesarean Section Scar Pregnancies

Although the European Society of Human Reproduction and Embryology (ESHRE) classified CSSP as one of the uterine EPs,[15] other authors classify CSSP as an IUP because it may result in live offspring if not terminated.[16] CSSPs occur following implantation of the fertilised oocyte over the previous CS or hysterotomy scars.[16] The number of scars is not a risk factor for CSSP, but it occurs following elective CS, which is explained by the impaired healing of an unprepared lower uterine segment.[2]

Clinical Presentation of Extrauterine Pregnancies

The triad of secondary amenorrhoea, first trimester spotting, and pelvic pain may occur with EPs as well as intact IUPs and early miscarriages. Further suggestive manifestations of EPs include pain on movement of the cervix, acute abdominal pain radiating to the shoulder(s), abdominal guarding, hypovolaemic shock, and syncope. Differential diagnosis of EPs includes complicated ovarian or adnexal cyst, tubo-ovarian abscess, appendicitis, and ovarian hyperstimulation syndrome.[1]

DIAGNOSIS OF EXTRAUTERINE PREGNANCIES

β-human Chorionic Gonadotropin

Suspicion of an EP begins after a positive pregnancy test and with the absence of definite IUGS by ultrasound; i.e., pregnancy of unknown location (PUL). Definite IUGS means IUGS with yolk sac and/or embryo.[1,13] The diagnosis of PUL will be changed to IUP in approximately 30% of cases after appearance of definite IUGS by ultrasound, while the majority of PUL (50–70%) will be changed to either miscarriages or EPs. β-hCG is produced by the syncytiotrophoblasts, detected in the blood by the second week of pregnancy, and its measurement is crucial to clarify pregnancy location and prognosis.[2] A single β-hCG assay is insufficient to detect early pregnancy prognosis, and serial β-hCG are commonly used to monitor early pregnancies.[3]

The recommendations of β-hCG rise came from retrospective review of PUL, which suggested a minimum β-hCG rise of ≥35% in 48 hours to diagnose IUP.[3] A β-hCG rise <35% in 48 hours has 96.2% PPV, 69.7% negative predictive value, and 80.2% overall accuracy in predicting EPs.[3] EPs are generally associated with a rise in β-hCG by no more than 66%, or a fall by no more than 13% from the baseline level, in 48 hours. The combination of β-hCG ratio lying within this range, along with initial β-hCG >1,500 mIU/mL in the absence of definite IUGS, has 92% sensitivity and 84% specificity in predicting EPs.[17]

Serum Progesterone

A meta-analysis concluded that a serum progesterone level of <10 ng/mL predicts non-viable pregnancy with 66.5% sensitivity and 96.3% specificity.[18] Serum progesterone is not useful in predicting EPs and cannot differentiate EPs from early miscarriages.[19]

Discriminatory Zone

The discriminatory zone is the serum β-hCG level at which definite IUGS can be seen by the TVS, confirming the diagnosis of IUP (≥1,000–2,000 mIU/mL).[2] Despite the discriminatory zone, a review of PUL reported visualisation of IUGS confirming the diagnosis of IUPs in nine females with β-hCG >2,000 mIU/mL.[20] In multiple

pregnancies, definite IUGS(s) may be seen at β-hCG value higher than the discriminatory zone identified for singleton IUP.[2]

A positive pregnancy test in the absence of definite IUGS should be considered EP until proven otherwise. The risk of EP is much reduced (but not zero) after visualisation of IUGS because of the possibility of HP.[2]

Additional Ultrasound Findings

Additional ultrasound findings may be useful to increase the suspicion for EPs. IUPs have significantly higher endometrial thickness than miscarriages or EPs (17 mm versus 12 mm, respectively).[21] In 20% of tubal EPs, a collection of fluid within the uterine cavity could be seen, referred to as the pseudosac (pseudosac is not diagnostic for tubal EPs).[21] A small amount of fluid in the Pouch of Douglas may be found in both IUPs and EPs, while large amounts of free fluid, particularly in the Morrison's pouch, may indicate haemoperitoneum in ruptured EPs.[2] Visualisation of an adnexal gestational sac (GS) with yolk sac and/or embryo as an echogenic periphery and non-echogenic interior (bagel sign) with circumferential Doppler flow without definite IUGS is highly suggestive of EP.[1] If the visualised, suspicious GS is round, echogenic, and moves separately from the ovary, this is considered the 'blob sign'. The blob sign has >90% PPV in diagnosing EPs in symptomatic females with positive β-hCG and no definite IUGS by TVS.[2]

A systematic review concluded that 88% of tubal EPs were diagnosed by the combination of an absent IUGS with an adnexal mass.[2] Ultrasound criteria of IEPs occur following implantation of the fertilised oocytes in the interstitial portion of the FT when it traverses through the uterine muscular wall (1–2 cm) to enter the cavity, and is a separate entity from the cornual pregnancy. Cornual pregnancy describes pregnancy in the rudimentary horn of a uterus with Müllerian anomaly.[4-9] The ultrasound criteria of the IEPs include an empty uterus with an eccentric GS seen separate from the endometrium, and the GS >1 cm away from the most lateral edge of the uterine cavity and surrounded by <5 mm myometrium in all imaging planes. Interstitial line sign has 80% sensitivity and 98% specificity in diagnosing IEPs. Interstitial line sign is an echogenic line extending from the GS to the endometrium cavity, representing the interstitial portion of the FT.[4-9]

In the absence of definite IUGS, non-tubal EPs can be suspected by specific ultrasound criteria. Cervical EPs can be identified by an empty uterus, a soft and ballooned cervix (barrel-shaped cervix), the GS lying within the cervical canal below the closed internal cervical os with evident blood flow around the GS detected by the colour Doppler, or absence of the sliding sign.[13,23] The sliding sign is used to differentiate the cervical EPs from miscarriages within the cervical canal. When pressure is applied on the cervix by the transvaginal probe, the GS slides against the endocervical canal in miscarriage but it does not in cervical EPs.[13,23]

There are no specific ultrasound criteria for the ovarian EPs.[12] Ovarian EPs are suspected by an empty uterus, a wide echogenic ring with internal anechoic area seen in the ovary, evident blood flow surrounding the echogenic ring detected by colour Doppler, and ovarian EP movement with the movement of the ovary when pressure is applied by the transvaginal probe.[2] Ruptured ovarian EP could be seen by ultrasound as a complex adnexal mass with free fluid in the Pouch of Douglas.[12] It may be difficult to differentiate an ovarian EP from an ovarian cyst, and definite diagnosis can be confirmed at laparoscopy.[24]

AP can be either primary, following implantation of the fertilised oocyte into the peritoneal surface (rare), or secondary, following ruptured tubal EP or tubal abortion. The ultrasound suggestive criteria of APs include an empty uterus, lack of evidence of dilated FT or complex adnexal mass, a gestational cavity proximal to the anterior abdominal wall and surrounded by bowel loops, and no myometrium separating the gestational cavity from the urinary bladder.[25]

The diagnosis for primary AP diagnosed with laparotomy using the Studdiford's Criteria includes healthy tubes and ovaries with no evidence of recent or remote injury, no evidence of utero-placental fistula, and recent pregnancy related exclusively to the peritoneal surface.[26]

Ultrasound criteria of CSSPs include an empty uterus with an empty, closed endo-cervical canal, location of the GS in the lower anterior quadrant of the uterus below the bladder close to the internal cervical os and previous scar(s)

with yolk sac and/or embryo, a thin myometrial layer between the GS and urinary bladder, and numerous blood vessels and arterio-venous malformation around the GS.[16]

Laparoscopy is the gold standard for surgical treatment of EPs. Its role in diagnosing EPs decreased after the use of TVS and β-hCG.[14]

TREATMENT OF TUBAL EXTRAUTERINE PREGNANCY

Expectant Management

The ACOG reported spontaneous resolution of EPs in 88% of patients at initial β-hCG <200 mIU/ml following expectant management.[10] In addition, a retrospective study, reported 88% success rate following expectant management for EPs at initial β-hCG <200 mIU/ml, while the success rate was 25% success rate at initial β-hCG >2,000 mIU/l[27] (Table 1). The Royal College of Obstetricians and Gynaecologists (RCOG) recommends expectant management for tubal EPs at initial β-hCG <1,500 mIU/mL in clinically stable patients with decreasing β-hCG.[13]

Medical Treatment

Medical treatment of EPs using MTX is more cost-effective than surgical treatment, with similar success rates[29] (Tables 1 and 2).

Systemic methotrexate regimens

A systematic review showed that the success rate of single-dose MTX was 94.4% when the initial β-hCG was between 1,000 and 1,999 mIU/mL, and was 81.1% when the initial β-hCG was between 10,000 and 150,000 mIU/mL.[30] Two-dose MTX regimen has been proposed by Barnhart et al.[31] with 87% success rate. A retrospective study reported 87% success rate for the single-dose MTX regimen at initial β-hCG of 4,801 mIU/mL versus 90% success rate for the two-dose MTX regimen at initial β-hCG of 4,278 mIU/mL.[32]

A meta-analysis reported 89% overall success rate for multiple-dose MTX in treatment of EPs; the multi-dose regimen was significantly more successful than the single-dose regimen (93% versus 88%) but caused more side effects.[11]

Surgical Treatment

Laparoscopy is the gold standard for surgical treatment of EPs for fast recovery, minimal hospital stays, and cost.[1,2] The surgical methods used for treatment of tubal EPs are salpingectomy or salpingostomy (Table 1).[28] The decision of salpingectomy or salpingostomy depends on the contralateral FT status and desired future fertility.[14]

TREATMENT OF INTERSTITIAL ECTOPIC PREGNANCY

Treatment of IEPs is reliant on gestational age at diagnosis, whether the IEP is intact or ruptured, and desired future fertility. The non-surgical options can be used with intact IEP, while a ruptured IEP is a medical emergency that requires surgery.

Non-surgical Treatment

Non-surgical treatment includes expectant management, systemic MTX, and local injections[33,34] (Table 3). The risks of non-surgical treatment for IEPs include subsequent rupture and life-threatening haemorrhage.

Surgical Treatment

Surgical treatment is indicated with ruptured IEPs and when non-surgical treatment fails or is not feasible. Laparotomy has previously been the traditional route for surgical treatment of IEPs, particularly ruptured IEPs. Laparoscopic approach is now commonly used for surgical treatment of intact or even ruptured IEPs[33] (Table 3).

TREATMENT OF NON-TUBAL ECTOPIC PREGNANCY

Cervical Ectopic Pregnancy

Cervical EPs may be managed medically, surgically, or by combined approaches. The use of systemic and/or local MTX has been described in case reports of cervical EPs.[35] An 87% success rate was reported in a series of cervical EPs treated with local MTX and additional potassium chloride injections.[36]

Table 1: Treatment of tubal ectopic pregnancies.

Expectant management	ACOG: • Asymptomatic and reliable patients for follow-up • Low β-hCG 175–200 mIU/mL • Haemodynamically stable (no evidence of ruptured EP)[10]
	RCOG: • Expectant management for tubal EPs at initial β-hCG <1,500 mIU/mL after ultrasound diagnosis in a clinically stable patient[13]
	• Decreased β-hCG within 48 hours indicates successful expectant management
Medical MTX treatment	• MTX is a dihydrofolate reductase inhibitor that disrupts DNA and RNA synthesis, and targets the rapidly dividing trophoblasts[10] • The most common side effects of MTX are elevated liver enzymes (mild and transient), nausea, abdominal pain, dermatitis, and stomatitis • Females should be advised to cease alcohol consumption (which elevates liver enzymes) and folate supplementation (which counteracts MTX action) before MTX treatment
	Indication of MTX: • Haemodynamically stable and reliable patient for follow-up • Pre-treatment β-hCG 1,500–5,000 mIU/mL • Ectopic size <4 cm in largest diameter • No fetal cardiac activity • No concomitant IUP • No known sensitivity to MTX
	Before MTX therapy: • A blood sample for β-hCG assay, CBC, liver, and kidney functions tests should be taken. • A chest X-ray should be considered to exclude active pulmonary lesions, and Rh status to determine females in need for Rho(D) Ig • TVS to obtain the baseline ultrasound criteria of the EP and exclude concomitant IUP[10]
	Absolute contraindication of MTX: • Haemodynamically unstable patients • HPs (EPs with concomitant viable IUPs) • Elevated liver enzymes • Serum creatinine ≥1.5 mg/dL • Total leucocyte <1,500/mm^3 • Platelets <100,000/mm^3 • Moderate-to-severe anaemia • Current breastfeeding • Active pulmonary disease • Active peptic ulcer • Sensitivity to MTX[10]
	Relative contraindication of MTX: • Presence of fetal cardiac activity • β-hCG level >5,000 mIU/mL • An ectopic mass size >4 cm in largest diameter • Unreliable patient for follow-up[10]

Table 1 continued.

Surgical treatment	Indications: • Surgical management for EPs is indicated when the medical treatment fails or is contraindicated, or when ruptured EP is suspected
	Salpingectomy (removal of the FT):[28] • Recommended in cases of extensive tubal damage, rupture, prior tubal sterilisation, or a large tubal EP (>5 cm in diameter) • If the final histological examination of the FT after salpingectomy demonstrates evidence of a tubal gestation, no further assessment or follow-up β-hCG needed • PTT is more common after salpingostomy (7%) compared to salpingectomy (<1%).
	Salpingostomy (removal of the EP through a tubal incision):[28] • 1–2 cm linear incision made with electrocautery or scalpel over the bulging EP, the contents of the EP removed using forceps or hydro-dissection (hydro-dissection preferred to avoid incomplete removal and PTT) • The tubal incision can be left open to heal by secondary intention or sutured closed; a Cochrane review reported an insignificant difference between the two techniques regarding the rates of EP recurrence and subsequent IUP[28] • After salpingostomy, weekly β-hCG assay is necessary to rule out PTT • Single dose of MTX 24-hours post-operatively was reported to decrease the rate of PTT after salpingostomy

ACOG: American College of Obstetricians and Gynecologists; β-hCG: β-human chorionic gonadotropin; CBC: complete blood count; EP: ectopic pregnancy; FT: fallopian tube; HP: heterotropic pregnancy; IUP: intrauterine pregnancy; MTX: methotrexate; PTT: persistent trophoblastic tissue; RCOG: Royal College of Obstetricians and Gynaecologists; Rh: rhesus; TVS: transvaginal sonography.

A success rate of 61.5% was reported in a series of cervical EPs treated with systemic MTX.[37] Gestational age >9 weeks, β-hCG >10,000 mIU/mL, positive fetal cardiac activity, and crown–rump length >10 mm were associated with high failure rates of MTX in treatment of cervical EPs.[13]

Dilation and curettage is not recommended as first-line treatment because of bleeding risks (40% hysterectomy rate reported following dilation and curettage for cervical EPs).[2] Placement of cervical sutures, intracervical balloon tamponade, and/or pre-operative feticide injection were reported to minimise the bleeding risks during the management of cervical EPs.[38] Uterine artery embolisation has been reported as a prophylactic measure prior to cervical EP treatment or as an emergency treatment.[39]

Ovarian Ectopic Pregnancy Treatment

Ovarian EP treatment is primarily surgical, and laparoscopy is the gold standard for haemodynamically stable ovarian EPs.[12,13] Resection of the ovarian EP and conservative ovarian surgery are usually the main surgical objectives, particularly in females desiring future fertility.[12,13]

Successful treatment of ovarian EPs using systemic MTX at initial β-hCG up to 5,201 mIU/mL, as well as local MTX injection at initial β-hCG up to 12,075 mIU/mL, has been reported.[40,41]

Intramural Ectopic Pregnancy Treatment

Intramural EP treatment is dependent on the patient's condition at the time of diagnosis. Reported cases of intramural EPs in the literature have been managed surgically and sometimes with hysterectomy following ruptured intramural EP.[2] Recent reports described the laparoscopic treatment of intramural EPs.[42] In clinically stable patients, medical treatment of intramural EPs using systemic MTX was successful even with high β-hCG up to 25,140 mIU/mL.[43] Successful

management of intramural EPs using local MTX, potassium chloride injections at initial β-hCG of 74,872 mIU/mL,[44] as well as uterine artery embolisation at initial β-hCG level of 12,250 mIU/mL,[45] have been reported.

Abdominal Pregnancy Treatment

Advanced APs should be managed by laparotomy and a multi-disciplinary team.[26] After delivery of the fetus, the placenta should be left *in situ* if attached by major vessels or vital structures, followed by multi-dose systemic MTX, antibiotics, and follow-up. Attempts to remove the placenta may precipitate severe haemorrhage.[26] Pre-operative MRI defines the placental implantation site.[26] Intra-operative massive bleeding from the placental site can be controlled by interlocking sutures and/or packing. Packing and/or retained *in situ* placenta associated with risks of ileus, peritonitis, and abscess formation may necessitate a second-look laparotomy.[26] A meta-analysis reported high rates of blood transfusion with hepatic (46%) and retroperitoneal (40%) APs.[46]

TREATMENT OF HETEROTOPIC PREGNANCIES

A review of the literature showed that most HPs (72.5%) occur in the FT, while seven cases were cervical and three cases were CSSPs.[47]

Tubal Heterotopic Pregnancies

Medical treatments of tubal HPs include local potassium chloride or hyperosmolar glucose injections. More than 50% of the tubal HPs managed with local potassium chloride injections require subsequent salpingectomy.[48] Treatment of HPs with local and/or systemic MTX is contraindicated in the presence of viable IUP.[2,13] Surgical management has been described more frequently with tubal HPs because most of the cases presented with tubal rupture.[49]

Table 2: Systemic methotrexate regimens.

Single-dose MTX regimen Menon S et al., [30] 2007	• 50 mg/m² MTX, IM injection • β-hCG level should be checked on Days 4 and 7 following MTX dose • Decreased β-hCG by ≥15% on Day 7 indicates successful MTX treatment • Increased β-hCG level or <15% drop on Day 7 indicates second dose MTX needed • The success rates of MTX in treatment of EPs are high with low initial β-hCG levels
Two-dose MTX regimen Barnhart KT et al., [31] 2007	• Two-dose MTX regimen has been proposed by Barnhart et al., with 87% success rate and minimal side effects[31] • A retrospective study reported 87% success rate for the single-dose MTX regimen in treatment of EPs at initial β-hCG of 4,801 mIU/mL versus 90% success rate for the two-dose MTX regimen at initial β-hCG of 4,278 mIU/mL[32]
Multiple-dose MTX regimen Barnhart KT et al.,[11] 2003	• The multiple-dose MTX regimen derived from the use of MTX in treatment of GTDs, alternating with leucovorin (folinic acid to counteract the MTX side effects) • MTX (1 mg/kg body weight) on Days 0, 2, 4, and 6 alternating with leucovorin (folinic acid 0.1 mg/kg) on Days 1, 3, 5, and 7 combined with follow-up by β-hCG until the β-hCG falls by >15% from its peak value • Regardless of which MTX regimen used, if the β-hCG level does not decline adequately (<15% on Day 7) or increases, surgical management should be considered • If a patient's serum β-hCG declines adequately (≥15% on Day 7), no further intervention is required and the β-hCG level should be monitored weekly until it reaches non-pregnant level • β-hCG usually returns to normal (non-pregnant) level within 2–3 weeks

β-hCG: β-human chorionic gonadotropin; GTD: gestational trophoblastic disease; IM: intramuscular; MTX: methotrexate.

Table 3: Treatment of interstitial ectopic pregnancies.

Non-surgical treatment	
Expectant management	• Appropriate for asymptomatic IEPs with low and/or decreasing β-hCG[33,34]
Systemic MTX	• The success rate of systemic MTX in treatment of IEPs was 80% even with high β-hCG levels (106,634 mIU/mL) and presence of fetal cardiac activity • Surgery for ruptured IEP or rising β-hCG may be required for 10–20% of IEPs treated with systemic MTX • Close follow-up is necessary, and hospitalisation is rarely needed except for females with uncertain diagnosis • Many studies support the multi-dose systemic MTX regimen for treatment of IEPs[4,6]
Local injections	• MTX (most common), while 20% potassium chloride is preferred in IEPs associated with concomitant viable IUPs • Injection can be carried out via laparoscopic, hysteroscopic, or ultrasound-guided techniques • The success rate of local MTX in treatment of IEPs was 91% in one case series and 100% in other cases series[33,34]
Surgical treatment	
Transcervical suction evacuation	• Under laparoscopic or ultrasound guidance • Although this technique minimises the risk of uterine perforation at the cornua, there is minimal data about the effect of this technique on the tensile strength of the cornua, tubal patency, and future fertility[33]
Cornuostomy/ salpingostomy (can be done when the IEP ≤4 cm in diameter)	• Cornuostomy is equivalent to the linear salpingostomy technique that is used for surgical treatment of tubal EPs • The pregnancy is removed without removing the surrounding myometrium and the defect is closed after achieving haemostasis • Salpingostomy is a variation of the cornuostomy in which the incision is made at the insertion point of the FT into the uterine fundus, which allows removal of the trophoblastic tissue through this defect (done when the IEP diagnosed at early gestation)[33]
Cornual resection ([excision] can be completed when the IEPs >4 cm in diameter)	• The IEP and the surrounding uterine cornua are excised en-bloc through a circumferential incision followed by suture closure of the myometrium after achieving haemostasis • Diluted vasopressin can be injected into the myometrium around the IEP to minimise the blood loss during surgery[33]
Cornual wedge resection	• Cornual wedge resection by laparotomy was the standard surgical treatment for IEPs before laparoscopy • Cornual wedge resection involves en-bloc removal of IEP, and the surrounding myometrium
Mini-cornual excision	• Described by Moawad et al.[33] as an elliptical incision along the long axis of the IEP in the thinned-out myometrium through which the IEP is evacuated to preserve the architecture and vascularity of the uterus • The IEP base was intact, sometimes cauterisation is needed to achieve haemostasis • No myometrial defect was noted, and no sutures were needed[33]
Hysterectomy	• Previously the standard of care to treat 50% of IEPs • It is not commonly used nowadays as a treatment option for IEPs except in cases complicated with life-threatening haemorrhage

β-hCG: β-human chorionic gonadotropin; EP: ectopic pregnancy; FT: fallopian tube; IEP: interstitial ectopic pregnancy; IUP: intrauterine pregnancy; MTX: methotrexate.

Salpingectomy is preferable to salpingostomy in tubal HPs because the persistent trophoblastic tissue cannot be monitored in presence of ongoing IUP.[2]

Expectant management, hyperosmolar glucose injections, and cornual resection have been reported for management of interstitial HPs with successful live births.[50]

Non-tubal Heterotopic Pregnancies

Expectant management, local injections (potassium chloride or hyperosmolar glucose), suction curettage, or hysteroscopic resection have been reported for management of cervical HPs with successful live birth. A live birth rate of 80% was reported in a series of cervical HPs for which cervical cerclage was used to minimise the bleeding after the intervention.[51]

At the time of writing, three cases of live births have been reported after local potassium chloride injection in APs.[52] Local hyperosmolar glucose injection and laparoscopic wedge resection were reported for treatment of ovarian HPs with successful live birth.[53] Surgical intervention of ovarian HPs carries the risk of interrupting the hormonal support for the co-existing IUP from the corpus luteum.

TREATMENT OF CAESAREAN SECTION SCAR PREGNANCIES

CSSP is a type of IUP, and it may result in a live offspring if not terminated.[16] To date, there have been 27 live births reported following conservative management of CSSPs.[13] For CSSPs with no yolk sac, and/or fetal cardiac activity, the ultrasound and biochemical follow-up are sufficient until the serum β-hCG returns to non-pregnant levels with or without MTX.[16] For CSSPs with yolk sac and/or fetal cardiac activity, there are two treatment options: termination or continuation of the CSSP. Females who decide to continue the pregnancy should be counselled regarding the risks of haemorrhage, uterine rupture, morbid adherent placenta, and emergency hysterectomy.[16] To date, there have been 22 emergency hysterectomies reported for life-threatening haemorrhage and morbid adherent placenta following CSSPs.[13]

Termination of Caesarean Section Scar Pregnancies

Termination of CSSPs should be individualised based on the patient's age, desired future fertility, and clinician's experience.

Surgical approaches

Suction aspiration is the traditional choice for surgical approach but it exposes vessels to injury and is associated with major bleeding that may necessitate life-saving hysterectomy. Insertion of a Foley's catheter at the CSSP site, inflated with saline as compression tamponade, is a potentially useful approach, which can be combined with ultrasound-guided suction aspiration.[16] Traditional dilation and curettage is often complicated by haemorrhage (76% required further treatment, and 14% required hysterectomy in a series of CSSPs).[54]

Hysteroscopic bipolar resection has been reported for CSSP management at initial β-hCG up to 28,333 mIU/mL.[55] Hysteroscopic resection is not preferred for CSSP management when the residual myometrium is <3 mm because of uterine perforation and bladder injury risks. Trans-abdominal excision of CSSPs (laparotomy or laparoscopy) allows revision of the lower uterine segment and reduces recurrence risks. Laparotomy is indicated in CSSPs if complicated by life-threatening haemorrhage or suspected uterine rupture.

Local injections

Ultrasound-guided local MTX or potassium chloride injection is the most effective treatment for CSSPs between 6 and 8 weeks; it stops the cardiac activity immediately and should be considered when future fertility desired.[16]

Systemic Methotrexate Regimens

Several authors have supported the multi-dose systemic MTX regimen for CSSP treatment combined with ultrasound and β-hCG follow-up until the β-hCG returns to non-pregnant levels. Persistent trophoblastic tissue may occur following medical and/or surgical treatments of CSSPs except for hysterectomy.[16]

PERSPECTIVES AND CONCLUSION

Recurrence of Extrauterine Pregnancies

The recurrence of EPs is not affected by the treatment modality. The ESEP trial showed that the EP recurrence was similar following salpingostomy or salpingectomy (8% and 5%, respectively).[56] The reported recurrence rate following prior IEPs was 9.4%.[57] One case of recurrent cervical EP reported in a series of cervical EPs was treated with different modalities.[58] The data are insufficient to comment on the recurrence rates following prior ovarian, intramural EPs, or APs.

Future Fertility After Extrauterine Pregnancies

Conception is not recommended for 3 months after MTX therapy. IUP rates are similar with no significant difference following salpingostomy or salpingectomy in the ESEP and DEMETER trials.[56,59] In addition to this, IUP rates within 2 years after prior tubal EP were similar among salpingectomy (67%), salpingostomy (76%), and medical treatment (76%) in a population-based study.[60] Institutes and healthcare providers should follow clear guidelines and protocols for management of EPs. Institutes should implement competency-directed training programmes to increase healthcare providers' skills to diagnose and treat EP variants using different modalities.

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D-chiro-inositol, Vitamin D, and Epigallocatechin Gallate Avoid Surgery in Females with Uterine Fibroids: Two Case Reports

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Abstract

Uterine fibroids (UF) represent the most common benign tumours in females of reproductive age, and can negatively affect fertility. Patients with UFs need to reduce the tumour size with pharmacological treatments or surgically remove the fibroid before using assisted reproductive technology (ART). On the other hand, surgery implies long waiting times before ART to avoid the risk of rupture of the uterus. Long waiting periods are often unacceptable for older individuals who want to undergo ART procedures. Unfortunately, no specific and safe treatment for UFs is currently available. Here the author reports two cases of patients with UFs and associated heavy menstrual bleeding who seek pregnancy through ART. Both underwent a daily treatment with epigallocatechin gallate, vitamin D, vitamin B6, and D-chiro-inositol for 3 months. The patients showed a volume reduction of 73.8% and 68.4%, respectively. This was associated with decreased blood loss (42.1% and 48.7%, respectively). After 3 months from the end of the treatment, both patients underwent ART procedure without the need for surgical intervention.

INTRODUCTION

Uterine fibroids (UFs), also known as leiomyomas or myomas, are gynaecological tumours typical of reproductive age.[1] They rarely appear before menarche and usually regress after menopause.[2] Symptoms are present in approximately 50% of patients and mainly include heavy menstrual bleeding, which could lead to anaemia in some cases. Other symptoms depend on the volume and position of the tumour and comprise dyspareunia, pelvic pain associated with a feeling of pressure in the abdominal cavity, constipation, and urinary incontinence. The International Federation of Gynecology and Obstetrics (FIGO) classified UFs based on their location in the uterus: submucosal, pedunculate submucosal, intramural, subserosal, and five other intermediate categories. Submucosal UFs negatively affect fertility as well as intramural UFs when above 4 cm, even without cavity distortion. On the other hand, subserosal UFs have little or no effect on fertility.[3]

Today, the management of fibroids in those wishing to conceive is primarily surgical. However, surgery may cause pelvic and

intrauterine adhesions, damaging uterine integrity. For this reason, waiting 6–12 months post-surgery is generally suggested in view of using assisted reproductive technology (ART). Such delay may represent an obstacle for elderly people approaching ART, decreasing their chances to achieve and sustain pregnancy. Hence, surgical removal of fibroids for infertility must be undertaken only when there is evidence to support the improvement in pregnancy outcomes.[4]

Pharmacological treatments are meant to reduce the symptoms and the tumour size in preparation for surgery or, possibly, to avoid the procedure altogether. They include progestogens, androgens, aromatase inhibitors, oestrogen receptor antagonists, selective progesterone receptor modulators, and gonadotropin-releasing hormone agonists.[5] While many of these are used off-label, ulipristal acetate is the only selective progesterone receptor modulator with a specific indication for the treatment of UFs.[6] However, ulipristal acetate was withdrawn from the market in September 2020 because of severe side effects, including liver failure.[6,7]

Recently, vitamin D and epigallocatechin gallate (EGCG) have shown promising results against UFs both *in vitro* and *in vivo*.[8,9] Vitamin D deficiency has been correlated with higher risk of UF development as well as with higher UF volumes.[10] Moreover, vitamin D has shown efficacy in inhibiting UF growth and in improving patients' quality of life.[9] EGCG is the most abundant and biologically active catechin from green tea, accounting for at least 50% of the total catechin content in green tea leaves.[11] Several *in vitro*, *in vivo*, and clinical studies have shown multiple EGCG anti-cancer activities, including anti-proliferative, pro-apoptotic, anti-angiogenic, and anti-invasive functions.[12] EGCG has shown the same activities on UFs, leading to tumour volume reduction and an improvement in the quality of life.[8]

UFs originate from the uterine smooth muscle tissue (myometrium), and their growth depends on oestrogen and progesterone.[13,14] Worthy of note, the aromatase enzyme is over-expressed in UF tissue, allowing for autocrine production of oestradiol that facilitates tumour growth.[13]

D-chiro-inositol (DCI) is a cyclic polyalcohol belonging to the inositol family. Inositols are present in nine different stereoisomers, of which myo-inositol (MI) is the main abundant in nature, directly followed by DCI.[15] Inositol is the precursor of inositol triphosphate, which acts as second messenger in all the pathways involving G-protein-coupled receptors such as insulin, follicular stimulating hormone, and thyroid-stimulating hormone.[16] MI and DCI result effectively in the management of hormonal and metabolic alteration of polycystic ovary syndrome thanks to their activity as insulin second messengers.[17] Despite their similar molecular shape, they play different roles even when involved in the same pathways. Indeed, these two molecules act as insulin second messengers: MI driving the intracellular glucose intake while DCI stimulates glycogen synthesis. For this reason, their ratio is specific and regulated for each organ and tissue.[18] Recently, DCI showed efficacy in downregulating, in a dose-dependent manner, the mRNA expression of the aromatase enzyme in granulosa cells.[19] This effect prompted the investigation of DCI applications in oestrogen-dependent pathologies, including UFs.

Here the authours report two cases of females with UF treated with DCI, vitamin D, and EGCG, in order to define a novel and safe non-pharmacological treatment to avoid surgery before ART.

CASE DESCRIPTION

Two females with UF, Case 1 and Case 2, were recruited between March and September 2020 and gave their oral informed consent after the explanation of the study purpose. This study was conducted following the Ethical Principles of the Helsinki Declaration and the national laws. Both patients were seeking pregnancy through ART.

The patients underwent a treatment consisting of 150 mg epigallocatechin gallate, 1,000 IU vitamin D, 5 mg vitamin B6, and 50 mg DCI (Delphys® plus, Farmares Srl, Rome, Italy) once per day for 3 months. This dosage was chosen because of the previous clinical data of the study of Porcaro et al.,[25] which obtained significant results in reducing UF volume and controlling symptoms in the absence of side effects. Transvaginal 2D images of the uterus were obtained both in the mid-sagittal

and transverse planes by a single observer using a Voluson™ E8 (GE Healthcare, Chicago, Illinois, USA) and a 7.5 MHz transvaginal probe. The 3D volumetric acquisitions were obtained for each UF on the mid-sagittal plane. Measurements of the fibroid in mm (anterio-posterior, longitudinal, and transverse) were recorded in ViewPoint™ (GE Healthcare). The 3D data were reviewed using SonoView Pro® (Medison Ltd., Seoul, South Korea) and UF volumes were calculated using virtual organ computed-aided analysis (VOCAL). The 3D volumetric measure was re-opened using SonoView Pro software and the outline of the UFs was traced manually using VOCAL, with six steps of rotation, 30° apart. After manual tracing, the VOCAL programme automatically displayed the 3D reconstructed fibroid with its volume. The patients were evaluated at baseline, after 3 months of treatment (T1), and at follow-up, performed after a further 3 months without any treatment (T2).

Case 1

A 38-year-old female seeking pregnancy was referred to the authors' fertility centre. The patient, with a BMI of 21.3 kg/m^2, reported an intense blood loss and transvaginal sonography showed an intramural UF with a volume of 164.3 cm^3. The UF led to uterine cavity distortion and the patient was eligible for surgery in view of the ART procedure. The patient reported an increase in serum vitamin D content from baseline (16.3 ng/dL) to the end of the treatment (24.1 ng/dL). The vitamin D serum level did not report a relevant variation at T2 (23.8 ng/dL). At baseline, the patient answered a standard semi-quantitative pictorial questionnaire for the evaluation of blood loss,[1] showing an estimated blood loss of 85 mL. Although the heavy bleeding reduced red blood cell (RBC) count, with a value of 3.8 millions/mm^3, the patient showed a normal haemoglobin (Hb) level (12.61 g/dL). At T1, a 70.96% reduction of UF volume was observed (47.7 cm^3), accompanied by a 42.1% reduction of bleeding (50 mL). Despite the blood loss reduction, RBC and Hb did not show notable improvement, with respective values of 3.8 millions/mm^3 and 12.80 g/dL. At T2, the patient underwent further evaluation after 3 months without any treatment. The results indicated a modest volume and bleeding increase, compared to T1 (21.54% and 10.00%, respectively). Volume reduction and blood loss are reported in Figure 1 and Figure 2, respectively. On the other hand, the RBC count increased to 12.81 g/dL, while the haemoglobin remained unchanged (12.72 g/dL). These results are reported in Table 1. After the study period, the patient did not need surgical intervention before ART.

Case Two

A 39 year-old female seeking pregnancy was referred to the author's fertility centre. The patient, with a BMI of 23.4 kg/m^2, reported an intense blood loss and transvaginal sonography showed an intramural UF with a volume of 77.9 cm^3. The UF led to uterine cavity distortion and the patient was eligible for surgery in view of the ART procedure. The patient reported an increase in serum vitamin D content from baseline (14.2 ng/dL) to the end of treatment (19.8 ng/dL). The vitamin D serum level did not report a relevant variation at T2 (20.1 ng/dL). At baseline, the patients answered a standard semi-quantitative pictorial questionnaire for the evaluation of blood loss,[1] showing an estimated blood loss of 92 mL. Although heavy bleeding reduced RBC with a value of 3.25 millions/mm^3, the patient showed a normal haemoglobin level (11.1 g/dL). At T1, a 65.98% reduction of UF volume was observed (26.5 cm^3), accompanied by a 47.8% reduction of bleeding (48 mL). Despite the blood loss reduction, RBC and Hb did not show notable improvement, with respective values of 3.75 millions/mm^3 and 11.90 g/dL. At T2, the patient underwent further evaluation after 3 months without any treatment. The results indicated a modest bleeding increase despite the absence of volume increase, compared to T1 (18%). Volume reduction and blood loss are reported in Figure 1 and Figure 2, respectively. On the other hand, the RBC count increased to 11.93 g/dL while the haemoglobin remained unchanged (11.57 g/dL). The results are reported in Table 1. After the study period, the patient did not need surgical intervention before ART.

DISCUSSION

In these two cases, the combination of EGCG, vitamin D, vitamin B6, and DCI avoided surgical intervention before undergoing ART procedure. In fact, at the end of the treatment, both patients showed an important UF volume and bleeding

reduction, and surgery was no longer necessary. Although the period of treatment was insufficient for restoring the RBC level, a normal erythrocyte count was obtained on the second follow-up according to the erythropoietic capacity, which requires 12–16 weeks. In previous studies, vitamin D and EGCG, alone and in combination, showed promising results in the management of UFs.[8] In the study of Ciavattini et al.,[10] vitamin D supplementation reduced the progression to severe symptomatology and, thus, the need for conventional surgical or medical therapy.

Table 1: Results of Case Studies 1 and 2.

	Baseline	T1	T2
Case 1			
Volume (cm^3)	164.3	47.7	60.8
Bleeding (mL)	85	50	55
Menstrual length (days)	6.0	5.5	5.5
Vitamin D serum level (ng/dL)	16.3	24.1	23.8
RBC (millions/mm3)	3.80	3.80	12.81
Hb (g/dL)	12.61	12.80	12.72
Case 2			
Volume (cm^3)	77.9	26.5	26.5
Bleeding (mL)	92	48	57
Menstrual length (days)	7.0	5.0	5.0
Vitamin D serum level (ng/dL)	14.2	19.8	20.1
RBC (millions/mm^3)	3.25	3.75	11.90
Hb (g/dL)	11.10	11.93	11.57

RBC: red blood cell; Hb: haemoglobin; T1: after 3 months of treatment; T2: follow-up performed after further 3 months without any treatment.

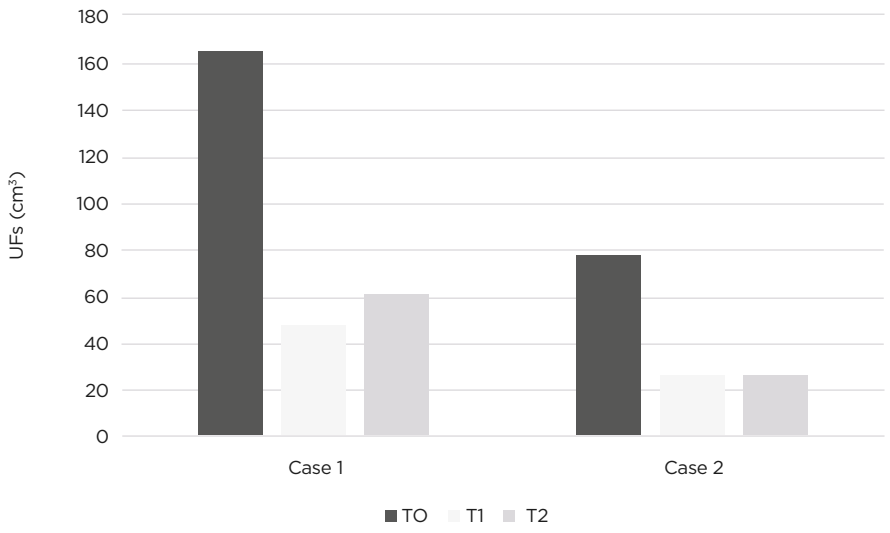

Figure 1: Uterine fibroid volume (cm^3) in Case 1 and Case 2 at baseline (T0), after the end of the treatment (T1), and after 3 months of follow-up without any treatment (T2).

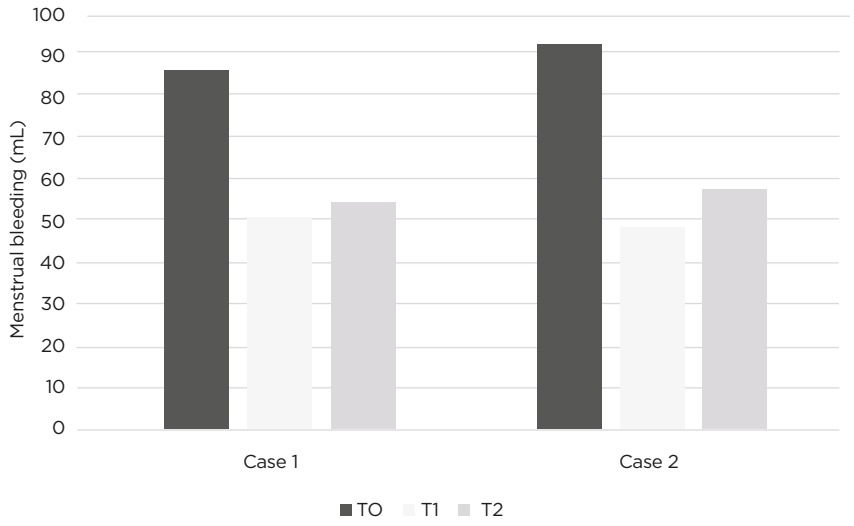

Figure 2: Menstrual bleeding (mL) in Case 1 and Case 2 at baseline (T0), after the end of the treatment (T1), and after 3 months of follow-up without any treatment (T2).

In particular, vitamin D showed significant growth inhibition compared with the control, which showed an increase in UF volume as well as worsening of the symptomatology.[10] Although vitamin D did not lead to UF volume reduction in the Ciavattini et al. research, recent studies report that vitamin D can reduce UF size both *in vitro* and *in vivo* via the suppression of cell growth and proliferation-related genes.[20,21]

In particular, vitamin D showed efficacy in inhibiting proliferation in UF cell line (human uterine leiomyoma cells) through the downregulation of proliferating cell nuclear antigen and cyclin-dependent kinase 1, a crucial protein involved in cell cycle regulation. In the same study, vitamin D demonstrated a pro-apoptotic effect by reducing the expression of B-cell lymphoma 2.[22]

In recent years, EGCG also proved effective in the treatment of UFs.[8,23-26] Its activity has been demonstrated both *in vitro* and *in vivo*, showing anti-proliferative and pro-apoptotic effects. Patients treated with oral EGCG reported UF volume reduction as well as improvement in symptomatology.

EGCG, like vitamin D, showed anti-proliferative and pro-apoptotic pathways through the downregulation of proliferating cell nuclear antigen, cyclin-dependent kinase 4, and B-cell lymphoma 2.[25-27] These effects were also demonstrated in the nude mouse model after the inoculation of the UF cell, with a significant tumour size reduction.[26]

In the study of Porcaro et al.,[24] the combination of vitamin D and EGCG has been tested for the first time in females diagnosed with UFs. The results showed UF volume reduction and improvement of the symptomatology.[24]

The use of aromatase inhibitors in the management of UFs resulted in a significant tumour volume and symptom reduction.[28,29] However, a recent Cochrane review stated that the evidence is insufficient to support the use of aromatase inhibitor drugs in the treatment of UFs[30] because of the lack of studies and the significant side effects. DCI, in combination with MI in a 40:1 ratio, enhances the signal of insulin[17] in pathologies characterised by insulin resistance, such as polycystic ovary syndrome, showing high efficacy and a good safety profile. Moreover, the effect of DCI in downregulating aromatase expression[19] opens to a broad spectrum of applications for different therapeutic targets, including UFs. UF cells overexpress the aromatase enzyme, producing oestradiol and sustaining their own proliferation.[13,31] In this regard, this is the first report of the use of DCI in the management of UF before using ART. The results suggest a high efficacy in reducing UF volume and menstrual bleeding, so that surgery was no longer necessary. The activity of DCI

on the aromatase might explain the important volume reduction reported in both cases. It is possible to speculate that vitamin D and EGCG exert their anti-proliferative and pro-apoptotic effects, enhanced by the activity of DCI.

The literature reports the correlation between UFs and fertility.[3] Submucosal, peduncular submucosal, and intramural UFs facing the internal uterine wall may deform the uterine cavity and alter the endometrium. As a consequence, gamete transport is impaired and the ability for embryo implantation reduced.[3] Surgery should be considered in infertile patients with fibroids before using ART.[32] However, surgery is associated with a 0.47% increased risk of uterine rupture in a following pregnancy in the short-term,[33] and ART procedures should be postponed. In the cases reported, treatment with DCI, vitamin D, and EGCG avoided surgical procedures and the need for a waiting period before ART. Randomised and controlled clinical trials are required to support these preliminary findings.

CONCLUSION

In conclusion, the combination of EGCG, vitamin D, and DCI shows high efficacy in reducing UF volume and, consequently, avoids the need for surgery. Such an approach represents an effective and safe alternative to other pharmacological treatments to avoid the delay before undergoing ART procedures but also for all those patients eligible for surgical treatment.

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