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EMJ Reproductive Health

Review of ESHRE Congress 2022

Editor's Pick

The Value of Serum Follicle-Stimulating Hormone in Predicting Successful Surgical Sperm Retrieval in Cases of Male Infertility: A Literature Review

Interviews

Mausumi Das and Signe Altmäe share insights into their fascinating career, and provide expert perspectives on the future of the reproductive health field

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Aims and Scope

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

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

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

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Front cover and contents photograph: Milan, Italy, home of the **ESHRE 2022 © ZacWolff** / unsplash.com

Welcome letter

Koutsouki

Evgenia Koutsouki
Editor

Dear Readers,

I would like to welcome you to the 2022 issue of *EMJ Reproductive Health*. This year's European Society of Human Reproduction and Embryology (ESHRE) 38th Annual Meeting was held in Milan, Italy, and we have the pleasure of bringing you the key highlights from this event.

Of interest from ESHRE 2022 was a live session where two leading experts debated reproductive rights in this millennium. Discussion focused on positive outcomes of the Sustainable Development Goals of the United Nations (UN), as well as challenges that are still encountered in rights to reproductive health today, and we are proud to be including a summary of this in our journal.

In addition to our congress content, we are delighted to feature interviews with two leading experts, who provide insights into their clinical research and discuss the latest developments in the field, including microbiome testing and improved oocyte cryopreservation techniques.

Further, this issue contains fascinating reviews, research articles, and case reports. Our Editor's Pick addresses the important issue of predicting the attainment of spermatozoa after testis biopsy in patients with azoospermia. You will also have the opportunity to read a topical review of progesterone receptor abnormalities involved in the pathogenesis of endometriosis. Also not to be missed is the article investigating the immediate perinatal outcome of females with maternal near-miss and those without near-miss morbidity. This is a dynamic area of research and the updates provided in this article are a valuable addition to the literature.

I hope you enjoy reading this issue and, as always, I would like to extend a big thank you to our authors, peer reviewers, and Editorial Board for ensuring the content maintains a high quality. We look forward to seeing everyone at ESHRE 2023, which will take place in Copenhagen, Denmark.

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Foreword

Dear Colleagues,

It is my pleasure to welcome you to the latest edition of *EMJ Reproductive Health*.

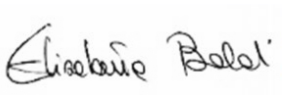
The 38th European Society of Human Reproduction and Embryology (ESHRE) meeting was finally in held in person after 2 years. It was held in Milan, Italy, in the beautiful Milano Convention Center (MiCo) and was very successful, with over 10,000 participants. All of the sessions were well organised, with a good level of science, and were followed by a very high number of participants.

As usual, the meeting was initiated by the most downloaded paper from ESHRE journals, which was held this year by Mauro Gacci, University of Florence, Italy, who presented the results of a study on male semen parameters in males recovering from COVID-19, showing how the illness may compromise testicular function; however, likely and fortunately, not permanently.

Many sessions this year were focused on male infertility, facing the important problem of ameliorating the diagnostic process of these males and evaluating sperm DNA fragmentation, sperm aneuploidies, and other markers that can be of great help. An interesting study by Pedro Melo, Tommy's National Centre for Miscarriage Research, University of Birmingham, UK, presented the results of a meta-analysis concerning 171 studies and almost 37,000 involved females ,who were treated with short stimulation regimens in order to reduce the risk of hyperstimulation. The study demonstrated that using long protocols is unlikely to improve live birth rates but will probably increase the risk of hyperstimulation, giving an important insight regarding treatment regimens for assisted reproductive technology procedures.

The session on pros and cons of artificial intelligence in assisted reproductive technology is also noteworthy. The interesting debate by Dean Morbeck (pros) from the Fertility Associates, Auckland, New Zealand, and Peter Tennant (cons) from the University of Leeds, UK, led to the conclusion that although artificial intelligence is certainly of great interest and help for embryologists, it is still in an 'embryo phase', and improvements are needed to render it more efficient.

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



Elisabetta Baldi

Associate Professor of Medical Pathology, Department of Experimental and Clinical Medicine, University of Florence, Italy

ESHRE 2022

Review of the European Society of Human Reproduction and Embryology (ESHRE) 38th Annual Meeting 2022

Location: Milan, Italy
Date: 3rd–6th July 2022
Citation: EMJ Repro Health. 2022;8[1]:8-16. DOI/10.33590/emjreprohealth/10143244. https://doi.org/10.33590/emjreprohealth/10143244.

THE 38th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE) took place in Milan, Italy, and online between 3rd and 6th July 2022. During the opening ceremony, Giovanni Coticchio, Immediate Past Chairman of the Special Interest Group (SIG) Committee, highlighted the important role Italy has played in ESHRE history, having contributed three society chairs as well as hosting three previous annual meetings.

As of 3rd July, there were 10,006 registered participants (82% in-person and 18% virtual) from 130 countries across the globe. Furthermore, 1,623 abstracts were submitted. Similar to previous years, embryology, andrology, reproductive endocrinology, and implantation and early pregnancy were the most popular abstract topics.

During his welcome address, ESHRE Chair Carlos Calhaz-Jorge highlighted the "rich and diversified" scientific programme. Indeed, symposia spanned across the discipline, with updates on the management of endometriosis, co- and pre-treatment for ovarian stimulation, novel aspects of recurrent pregnancy loss, fertility preservation in females with cancer, COVID-19 and implantation, new markers of male health and function, and the current state of time-lapse technology. Of note was the session debating reproductive rights in this millennium, which saw two leading experts discuss the positive outcomes of the Sustainable Development Goals of the United Nations (UN) as well as challenges that are still experienced in reproductive health today. This forms the basis of our compelling in-house feature and is not to be missed.

"Similar to previous years, embryology, andrology, reproductive endocrinology, and implantation and early pregnancy were the most popular abstract topics."

Alongside the impressive programme were the annual prizes for standout presentations. The Clinical Science Award for oral presentation was awarded to Christian Becker, Nuffield Department of Women's and Reproductive Health, University of Oxford, UK, for his report on relugolix combination therapy in females with endometriosis-associated pain. The Clinical Science Award for poster presentation was presented to Juan

"Read on for our key scientific insights from ESHRE's 38th Annual Meeting."

Giles, Reproductive Medicine, IVIRMA Valencia, Spain, for his presentation on medroxyprogesterone acetate as an alternative to gonadotropin-releasing hormone antagonists in oocyte vitrification for non-oncological fertility preservation and pre-implantation genetic test cycles. The work of Sara Somers, Department of Reproductive Medicine, Ghent University Hospital, Belgium, was celebrated when her presentation, discussing uniform communication by nurses and midwives in anticipation of *in vitro* fertilisation treatment, received the Nurses Award for best oral presentation by a nurse.

An overview of groundbreaking ESHRE press releases can also be found within this issue of *EMJ Reproductive Health*, including whether probiotics improve poor vaginal health, the association between anorexia and adverse pregnancy outcomes, and the effectiveness of frozen sperm for insemination treatments.

Calhaz-Jorge also spoke about some of the crucial activities carried out by ESHRE, such as the provision of education through the annual meeting, e-Learning platform, campus workshops, and webinars. ESHRE is also responsible for setting standards, including the recent evidence-based guidelines for endometriosis, medically-assisted reproduction in patients with a viral infection or disease, and female fertility preservation. There are also a range of good practice guidelines, which cover pre-implantation genetic testing, information provision for those involved in reproductive donation, and more. ESHRE is also collaborating with the patient organisation Fertility Europe to create a roadmap for fertility awareness and advocacy across Europe.

"The strength of our society is the membership," concluded Calhaz-Jorge. ESHRE now has 9,379 members from 135 different countries. In contrast, there were less than 8,000 members in 2019. "We crossed the pandemic gaining more than 1,000 new members," said Calhaz-Jorge. Clearly, conferences such as ESHRE are crucial for the generation and exchange of scientific knowledge. With this in mind, we look forward to being part of the international human reproductive health and embryology community again at next year's congress in Copenhagen, Denmark. Until then, read on for our key scientific insights from ESHRE's 38th Annual Meeting. ●

Can Probiotics Improve Unhealthy Vaginal Flora?

THE TYPE of bacteria that naturally colonise the reproductive tract are known to influence pregnancy chances in *in vitro* fertilisation. Specifically, in females with a vaginal microbiota dominated by *lactobacillus*, pregnancy and live birth rates are known to be higher. Conversely, among those with a lower *lactobacillus* concentration, the chance of an embryo implanting in the uterus is reduced. For this reason, there is increasing interest in the role of probiotics as a treatment for females with an imbalance in the vaginal microbiota.

A study presented at ESHRE's 38th Annual Meeting explored whether a probiotic containing strains of *lactobacilli* could improve unhealthy vaginal flora when administered vaginally in a daily capsule to patients for 10 days before fertility treatment.

The trial was conducted between April 2019 and February 2021 at a university fertility clinic. In total, 74 females referred for *in vitro* fertilisation were enrolled. All participants had a *lactobacillus* profile that ranged from low to medium quality. The females were randomly assigned to receive the capsules (n=38) or a placebo (n=36). Samples were taken to determine the effect on the vaginal microbiome at two time points: following the 10-day course of probiotics and in the subsequent menstrual cycle (between cycle days 21 and 25).

Overall, the vaginal microbiome was found to improve by 40% in the placebo group and 29% in females taking the *lactobacillus* probiotic. However, this did not represent a significant difference.

Primary investigator Ida Engberg Jepsen from The Fertility Clinic at Zealand University Hospital, Denmark, summarised the research findings: "The study indicates that administering vaginal *lactobacilli* products may not improve a suboptimal vaginal microbiome."

It is important to note that only two strains of *lactobacilli* were contained in the probiotic. Further, the authors indicated that the broad categorisation of the vaginal microbiome profile may not have captured the subtler changes that can affect fertility. Consequently, probiotics in general should not be discounted.

"A study presented at ESHRE's 38th Annual Meeting explored whether a probiotic containing strains of *lactobacilli* could improve unhealthy vaginal flora."

Also of relevance, 34% of all females who participated in the trial, regardless of whether they received the probiotic or placebo, exhibited an improvement in the vaginal microbiome between 1 month and 3 months later. Based on this, Jepsen highlighted an alternative therapeutic approach: "The strategy would involve postponing fertility treatment until spontaneous improvement occurs, but further research is needed." ●

Anonymity at Risk in Egg and Sperm Donations

COMMERICAL DNA testing is being used by individuals involved in donor conception to find information about genetic relatives. According to interim results from ConnecteDNA, a qualitative study presented at ESHRE's 38th annual meeting, individuals who are donor-conceived are using DNA testing to discover genetic family and their origins, including potential health risks.

A 2015 survey conducted by ESHRE found that 12 European countries had laws requiring anonymous egg and sperm donations; however, some countries had introduced non-anonymous schemes. For example, information on donors and individuals who were donor-conceived has been held in a register by the Human Fertilisation and Embryology Authority (HFEA) in the UK since 2005. Therefore, individuals conceived from donors can request identifying information about their donors when they turn 18 in 2023.

ConnecteDNA will continue into 2024, but the researchers have already performed interviews with 20 adults conceived through donors, 15 parents of children who are donor-conceived, and 14 donors. So far, the results indicate that commercial DNA testing is not just used for identifying a donor but also 'ethnicity estimates'. Meanwhile, some donors use these services to make themselves contactable to those conceived by their donation(s) and parents of children who are donor-conceived use it to trace genetic relatives.

"Individuals who are donor-conceived are using DNA testing to discover genetic family and their origins, including potential health risks."

However, commercial genetic testing and tracking family history from salvia samples matched against a DNA database means that there is no real anonymity for donors whose gametes have been used in donor conception, as they could have a close genetic relative who has used the service, making the donor traceable. Furthermore, there is a chance that a child who does not know that they were conceived through a donor could accidently learn of their origins, which could be distressing. Therefore, adequate support and counselling if unexpectedly exposed.

ConnecteDNA researchers will also examine legal regulations governing access and storage of donor information across the UK; the Netherlands; Victoria, Australia; and Sweden, with the aim to make regulatory recommendations. ●

Anorexia Nervosa Linked to Unfavourable Outcomes in Pregnancy

FEMALES diagnosed with anorexia nervosa are 500% more likely, on average, to give birth to underweight babies. Researchers found that the incidence of newborns born to mothers with anorexia who were born small for their gestational age was far higher when compared to babies born to females of a healthy weight.

A study, presented at ESHRE's 38th Annual Meeting, has discovered that there is a substantially increased risk of premature birth (298%), and more than double the chance of the mother experiencing placental abruption compared with mothers without anorexia.

The study was based on data collated from over 9 million females, both with and without anorexia. Records were taken from a large public-accessible database of hospital inpatient care documents across the USA. The study included all deliveries in the decade between 2004 and 2014, with a cohort of 214 females who had anorexia during their pregnancies, and more than 9 million females who did not.

Whilst eating disorders often have an impact on menstruation, females who have anorexia are able to conceive naturally, or with the assistance of fertility drugs to restore ovulation. Although marked differences in birth weights were recorded in this study, researchers did not find variance in other conditions that affect females who are pregnant, including gestational diabetes, post-partum haemorrhage, placenta previa, or hypertensive diseases. The necessity of performing a Caesarean section was also no greater in either cohort.

Researchers stressed that the risks associated with pregnancy in females diagnosed with anorexia need to be more widely understood by fertility specialists. Lead study author Ido Feferkorn, McGill University, Montreal, Canada, commented that the study's results carried a serious health message about the management of females with anorexia both during and following pregnancy. He remarked: "Clinics should be aware of the magnitude of adverse outcomes related to pregnancy among those patients with anorexia who do conceive."

"Current evidence suggests that the majority of healthcare professionals working in the field of reproductive health do not screen their patients for eating disorders."

Feferkorn advocated that, based on the wider implication of the study's findings, females should be screened for anorexia before beginning fertility treatment. Current evidence suggests that the majority of healthcare professionals working in the field of reproductive health do not screen their patients for eating disorders. ●

Timed Intercourse Assisted by Urine-Monitoring May Increase Changes of Pregnancy

DATA presented at ESHRE's 38th Annual Meeting has suggested that females may be able to increase their chances of conceiving by timing intercourse to 'fertile windows' by using urine-testing monitors. In recent years, timed intercourse has become more widely practised due to the surge in the availability of health apps focusing on ovulation detection.

The researchers emphasised that definitive conclusions could not be made about the efficacy of fertility tracking through other home-based methods such as fertility awareness-based methods (FABM), which are used in most menstrual tracking apps. FABM use calendar predictions and identify physiological changes to cervical mucus or body temperature with wearable detection devices to detect when ovulation is most likely.

The study focused on timed intercourse decided by urine monitors and urine ovulation tests as well as FABM. Data from six studies which included 2,374 females. The aim was to measure the impact this has on live birth rates, pregnancy rates, time of pregnancy, and overall quality of life. However, researchers were also interested in the negative impacts of timed intercourse, stress, lack of spontaneity, and pressure on sexual performance.

The authors found that the chance of pregnancy using timed intercourse, judged by urine-testing was between 20–28%. Whereas individuals who were practising spontaneous intercourse had comparably lower chance of pregnancy at 18%.

'This update suggests a benefit of timed intercourse using urinary ovulation detection. However, more evidence is needed on the adverse effects of timed intercourse and its effectiveness in different groups, before clinicians are able to promote this practice,' stated first investigator, Tatjana Gibbons, Nuffield Department of Women's and Reproductive Health, University of Oxford, UK.

"This update suggests a benefit of timed intercourse using urinary ovulation detection. However, more evidence is needed on the adverse effects of timed intercourse and its effectiveness in different groups."

In contrast, the study found no conclusive benefit of timed intercourse using FABM in live birth rate and pregnancy rates. However, the authors conceded that data was only available from two studies involving 160 females and, therefore, evidence was low grade.

Reducing the Risk of Ovarian Hyperstimulation Syndrome: Are Short Gonadotrophin Releasing Hormone Antagonists the Key?

META-ANALYSIS data from 171 randomised trials investigating the safety and efficacy of 56 different *in vitro* fertilisation (IVF) stimulation protocols, including almost 37,000 individuals, was presented by Pedro Melo, Tommy's National Centre for Miscarriage Research, University of Birmingham, UK, at the 38th Annual ESHRE Congress, 4th July 2022, Milan, Italy. The data reveals that use of short gonadotrophin releasing hormone (GnRH) antagonists reduces the risk of ovarian hyperstimulation syndrome (OHSS) and does not significantly affect live birth rate.

OHSS is a serious complication associated with use of exogenous gonadotrophins in IVF and occurs most frequently in patients who display a normal or high response to ovarian stimulation following gonadotrophin administration.

Previously, multiple gonadotrophin and GnRH agonist protocols have been trialled in order to improve outcomes and reduce risk. Traditionally pituitary downregulation has been achieved using GnRH agonists. This takes several weeks and is, therefore, referred to as 'long protocol'. However, it has recently been noted that using a GnRH antagonist leads to much faster pituitary downregulation. As such, these GnRH antagonists are referred to as 'short protocol'.

Data from this meta-analysis show that use of short GnRH antagonist protocols in patients with normal or high ovarian stimulation responses does not affect live birth rates and leads to a reduction in OHSS by up to 52%. The mechanism is not fully understood, but Melo stated that "it seems likely that this results from a combination of factors, including the absence of an ovarian 'flare' effect with GnRH antagonists". However, the meta-analysis findings do not include cumulative live birth rates, which are considered "the most effective measure of IVF success."

"Use of short GnRH antagonist protocols in patients with normal or high ovarian stimulation responses does not affect live birth rates and leads to reduction in OHSS."

Whilst severe OHSS is rare, affecting 1% of patients, mild OHSS is more common, affecting up to 33%. Symptoms occur secondary to ovarian enlargement and include abdominal distension and pain, and in rarer circumstances leads to serious cardiovascular, respiratory, and renal complications. Therefore, identifying methods to reduce OHSS risk is important. These findings are promising, and use of short GnRH antagonist protocols could lead to reduced patient harm. Further evaluation of their effect on cumulative live birth rates will provide more insight into their efficacy and impact of IVF success rates.

Frozen and Fresh Sperm Equally Are as Effective at Insemination

IN THE largest study of its kind, researchers have found that cryopreserved sperm is not associated with any inferior outcomes compared with fresh sperm in patients undergoing intrauterine insemination. The results, presented at ESHRE's 38th Annual Meeting, identified no difference in pregnancy rates between cycles using cryopreserved and fresh sperm.

In most jurisdictions, cryopreservation is the favoured method of sperm sample preservation. However, although its use is now widespread and is a requirement for some donor samples, patients still harbour concerns that the process of cryopreservation may reduce the viability of sperm with the freezing and thawing process impacting mobility, structure, and DNA content.

The study analysed 5,335 intrauterine insemination cycles performed at Massachusetts General Hospital, Harvard Medical School, Boston, USA, between 2004 and 2021, analysing a range of outcomes with either fresh or preserved sperm, including positive pregnancy test, clinical pregnancy, and miscarriage. The study was further controlled for the type of ovarian stimulation administered and whether it was administered or not.

The results of the study demonstrated that similar clinical pregnancy rates were found with both fresh and frozen sperm samples. The investigators noted some small differences in the sub-group analysis of patient having pre-treatment ovarian stimulation with oral medications; however, the differences were limited to the first cycle treatment and beyond this were negligible. The only notable, lasting difference was that time-to-pregnancy was slightly longer in the frozen sperm group than the fresh group.

"The fact that our data did not reveal any significant difference in success between the utilisation of fresh ejaculated and frozen sperm, except in a sub-group of patients given oral ovulation-inducing agents, is very reassuring to all involved," stated Panagiotis Cherouveim from Massachusetts General Hospital.

Cherouveim highlighted the importance of this research and the reassurance it should provide to single females and same-sex couples where cryopreserved sperm may represent the only opportunity for conception.

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Are Reproductive Rights Progressing in the 21st Century?

Author: Anaya Malik, Editorial Manager

Citation: EMJ Repro Health. 2022;8[1]:18-21. DOI/10.33590/emjreprohealth/10012502. https://doi.org/10.33590/emjreprohealth/10012502.

In a live session at the European Society of Human Reproduction and Embryology (ESHRE) 38th Annual Meeting, a pertinent debate over reproductive rights in this millennium was held between Jennifer Merchant, University Panthéon-Assas Paris II, France, and Marleen Temmerman, Aga Khan University Hospital, Nairobi, Kenya. The two leading experts, respectively, held a lively discussion on positive outcomes of the Sustainable Development Goals (SDG) of the United Nations (UN) versus the challenges that are still experienced in rights to reproductive health today.

UNITED NATIONS 21ST CENTURY MILLENNIUM DEVELOPMENT GOALS

Merchant initiated the discussion by explaining the eight international development goals were established by the Millennium Summit of the UN in 2000, to be achieved by the year 2015.[1] The declaration stated that every individual has a right to freedom and equality, among other key liberties. In 2010, 5 years prior to the target year, an evaluation meeting was held where co-ordinators identified that the intersectionality of gender equality meant that this goal should not be treated in isolation but should transverse all goals. New targets were established with a global strategy for women's and children's health, and the SDGs were replaced by the Millennium Development Goals (MDG). The new goals focused on women and children in poverty, hunger, disease, and health, and included the reduction of the maternal mortality ratio by three-quarters and the attainment of universal access to reproductive health. To reach this, Merchant confirmed that healthcare personnel had to increase in areas where there is a lack of them. Other notable actions included a pledge for prioritising larger access to reproductive health, especially availability of contraception, reduction of the adolescent birth rate, pre- and postnatal care, and centres of family planning through increased funding.

THE MILLENNIUM DEVELOPMENT GOALS: INDICATORS OF SUCCESS

Merchant argued that the increased focus on gender equality seemed to accelerate MDG progress in certain countries. In the 2010 review, it was reported that in South Asian countries, where there is low birth rate and high mortality due to immediate access to healthcare and malnutrition, there were larger numbers of women in paid and industrial work. This gave women better access to healthcare and better nutrition among the cohorts, which in turn reduced child mortality. Improved economic opportunities for women decreased participation in the sex market, which decreased the spread of AIDS and other sexually transmitted

infections. Also reported in 2010 was a study of women in rural Mexico, that showed that individuals who engaged in industrial work were able to negotiate and obtain a greater deal of respect in the home. In Tanzania, a study indicated that increased access to paid work led to a long-term reduction in domestic violence. Most evaluations and reports that were released by 2017 showed that at least 21 million lives were saved due to the accelerated progress of the UN's SDGs. Research on child mortality showed that 8.8–17.3 million children's lives were saved. Low-income countries improved more than middle-income countries. Merchant hypothesised that this could be explained by the 'failed states' in low-income countries, giving international organisations greater success in penetrating and applying programmes.

In conclusion, Merchant gave suggestions for the future of the MDGs set to be achieved in 2030. The progress made with the MDGs shows that putting emphasis on gender issues transversally has made an impact on the success of the goals. Notably, although the resources, technology, and knowledge exist to decrease poverty through improving gender equality, the will, often linked to religion, is still missing. Developing countries are advised to focus on priority areas applied to each of the goals to tackle this challenge, including girls' completion of secondary school and higher education, improving sexual and reproductive health rights, improving infrastructure to improve women and girls' time burdens, guaranteeing women's property rights, reducing inequalities in employment, increasing women's seats in government, and combatting violence and violence against women.

REPRODUCTIVE RIGHTS ARE COMPROMISED EVERY DAY

Temmerman launched into her discussion by giving an oversight of key milestones in the history of women's rights and gender equity. In the 1960s and 1970s, movements in Europe for access to contraception and for women to have a say in their own reproduction led to major achievements, including the legalisation of abortion in certain countries.

As an obstetrician-gynaecologist working in Kenya, Temmerman described her past experiences of reproductive health and gender inequality where she encountered many challenges, including lack of resources and technology, a shortage of materials, patients sharing beds, and commonly faced AIDS, and maternal and newborn mortality.

As explained by Temmerman, in 1994, the UN's International Conference on Populations and Development (ICPD) in Cairo, Egypt, was a landmark event in sexual and reproductive health as one of the first meetings to place women's reproductive rights within the human rights framework. For the first time in history, the participating countries agreed that reproductive health includes the right of a woman or adolescent to make their own reproductive choices. The Beijing Platform of Action was held in 1995 and attended by representatives from 189 governments, producing the most progressive influence ever seen for advancing women's rights. The Nairobi Summit ICPD+25 held in 2019, 25 years on from the original ICPD, reviewed the progress made. While the plan addressed critical areas of concern, Temmerman warned that resistance against reproductive rights from many countries around the world is still underestimated.

The major causes of maternal mortality can be identified as haemorrhage, hypotension, and septicaemia. These are reversible conditions that, as highlighted by Temmerman, can be managed by healthcare professionals via two key interventions: improving quality of care and family planning. She proposed that there would be a triple return on investment if countries prioritised quality of care at childbirth resulting in reduced maternal mortality, reduced newborn mortality, and prevention of stillbirths. Stillbirth rates are highest in low- and middle-income countries and stillborn babies commonly die during labour; indicating poor quality

of care in these areas. For example, there are still requirements for doctors in rural areas to perform gentle assisted pushing, or who are unable to check a baby's heartbeat.

"One in three pregnancy-related deaths could be avoided if all women had access to contraceptives."

Targeting family planning is also a key focus of achieving equal reproductive rights in the 21st century, according to Temmerman, who stated: "One in three pregnancy-related deaths could be avoided if all women had access to contraceptives." She also argued that progress regarding child and maternal mortality is slower than other developments made in the MDGs so far. Of 9 million deaths of women and children, 6 million relate to pregnancy and death. At the end of the 2015 goals, 303,000 women died during birth, 2.6 million babies were stillborn, 2.7 million newborn babies died, and 3.2 million children were stunted at birth.

The need for global discussions and development is still warranted. Every year, more than 200 million women try to avoid pregnancy but are not using modern contraception; over 45 million women receive inadequate antental care, or none at all; and more than 30 million women deliver their babies outside of healthcare facilities.

Advancements continue to be made as highlighted by Temmerman, including the Guttmacher-Lancet Report, which created a comprehensive definition of sexual and reproductive health rights; the work of societies such as the World Health Organization (WHO) and ESHRE to place infertility and reproductive rights high on conference agendas; and encouragement to adopt new policies and programmes at global, national, and regional levels.

In closing, Temmerman emphasised the support required to increase women's empowerment and progress in reproductive rights. She highlighted the need for more emphasis on gender issues within the MDGs, and stressed that action should be taken to collect further data on gender inequalities in order to achieve the UN's ambitious goals by 2030.

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Abstract Highlights

The following highlights cover several innovative and thought-provoking abstracts from the 38th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), featuring topics such as artificial intelligence in fertility treatments, a potential biomarker for endometriosis, and the use of testosterone therapy in *in vitro* fertilisation.

Could a Small Extracellular Vesicle Protein Cargo Be a Biomarker for Endometriosis?

ACCORDING to a study presented at the ESHRE 2022, which took place on 3rd–6th July, researchers identified that small extracellular vesicles (sEV) could be a biomarker for endometriosis. Females with endometriosis experience pain and subfertility; however, the cause of the disease is unknown. Although sEV are produced by nearly every cell, they have been identified in diseases such as cancer, pre-eclampsia, and diabetes, as well as endometriosis. The researchers for this study aimed to confirm if peritoneal fluid (PF) sEV could be utilised as a biomarker for endometriosis.

Samples of PF were acquired from the Endometriosis CaRe Centre, Nuffield Department of Women's and Reproductive Health, University of Oxford, UK. Females between 18–49 years old who had undergone diagnostic laparoscopy were organised according to their cycle phase, either proliferative, secretory, or menstrual, and according to the severity of endometriosis as per the American Society for Reproductive Medicine (ASRM) stages. The criteria for exclusion were based on ongoing hormonal treatment, malignancy, pregnancy, breastfeeding, and lack of ability to understand the consent form. In order to remove cells, debris, and microvesicles, 1 mL of PF from each sample was centrifuged. The sEV was isolated via size exclusion chromatography and investigated by a nanoparticle tracking analysis, immunoblotting, and mass spectrometry (liquid chromatography with tandem mass spectrometry).

> **"Researchers for this study aimed to confirm if peritoneal fluid (PF) sEV could be utilised as a biomarker for endometriosis."**

The results showed the existence of exosomes in PF in both females at different stages of endometriosis as well as participants without the disease. Additionally, sEV concentrations were higher in females with endometriosis compared to the control group and were highest in Stage III–IV endometriosis. The concentration of sEV in Stage III–IV endometriosis declined as the samples of PF shifted from proliferative to secretory cycle phases. Earlier studies have demonstrated CD44 as the sEV protein uniquely found in endometriosis, and this study aligned with this finding. In conclusion, the authors believe that the existence of CD44 within sEV could help diagnose endometriosis.

Viability of Embryo by the Use of Whole Genomic Amplification in Blastocoelic Fluid

A STUDY presented at ESHRE 2022, which took place on 3rd–6th July, stated that blastocysts with DNA in the blastocoelic fluid (BF) have lower ongoing pregnancy rates compared with blastocysts without DNA, both in preimplantation genetic screening (PGT-A) and in standard *in vitro* fertilisation (IVF) cycles. Several studies have reported the identification of DNA in BFs from expanded blastocysts; however, once the DNA is analysed to supply information on the blastocyst chromosome condition the results on the corresponding embryo ploidy have been variable. This could be due the contrasting status of the examined embryos. A study comparing euploid and aneuploid blastocysts recently reported a remarkably higher incidence of failed BF-DNA amplification in euploid blastocysts compared with aneuploid blastocysts implying that the outcome of the embryo ploidy condition to content in BF.

The prospective study, presented at ESHRE, included 142 cycles with PGT-A (Group 1; 24-chromosome analysis was performed on trophectoderm [TE] biopsies) and 121 standard IVF consecutive cycles (Group 2) treated in the last 3 years. In both groups, the BF was acquired from expanded blastocysts before vitrification and submitted to whole genomic amplification (WGA). Single blastocyst transfers were performed by selecting blastocysts based on BF-WGA results while prioritising those with failed amplification. In Group 1, only TE-euploid blastocysts was transferred. A total of 622 blastocysts underwent TE biopsy in Group 1 and 261 were euploid.

The BF was acquired from 237 euploid blastocysts, which were then submitted to the WGA for amplification. In total, 98 BFs experienced negative amplification and 139 BFs provided positive amplification. A total of 57 clinical pregnancies occurred, of which 53 pregnancies were ongoing. In terms of transfer outcomes, 61 transfers were performed with euploid blastocysts with failed BF-WGA and 81 with positive BF-WGA. The ongoing pregnancy rate was remarkable in the failed BF-WGA euploid blastocysts (50.8%) compared with those with positive BF-WGA (27.2%) in Group 1. Additionally, in Group 2, there was a total of 62 clinical pregnancies, with 52 being ongoing. The researchers observed the same ongoing pregnancy trend as Group 1 in terms of BF-WGA results.

> **"The researchers concluded that the DNA in BF could be a characteristic of an abnormal embryo attempting to achieve a viable state."**

Although this was a prospective cohort study and further research would be required from a prospective randomised trial, the researchers concluded that the DNA in BF could be a characteristic of an abnormal embryo attempting to achieve a viable state. Furthermore, if DNA is undetected following a BF, amplification could be considered as an extra criterion to determine viable embryos suitable for transfer in both in standard IVF cycles and PGT-A. ●

Could Artificial Intelligence Models Accurately Predict Outcomes in Fertility Treatment?

ARTIFICIAL INTELLIGENCE (AI) models could be used to assist clinicians in predicting outcomes in fertility treatment. The success of *in vitro* fertilisation (IVF) relies on accurate assessment of embryo quality. The limitation current methods to assess embryo quality is that they are subject to human error. Therefore, AI-based prediction models have been developed with the aim of improving the reliability of embryo grading.

A systematic review and meta-analysis of 18 published studies were presented at ESHRE 2022, which took place on 3rd–6th July 2022, by lead researcher Konstantinos Sfakianoudis, Centre for Human Reproduction, Genesis Athens Clinic, Assisted Conception Unit, Chalandri, Greece. He discussed the efficacy and accuracy of AI-based prediction models against four specific IVF outcome measures. The outcome measures assessed were prediction of clinical pregnancy, clinical pregnancy with heartbeat, live birth, and embryo ploidy status.

Of the 18 studies included, 10 reported on prediction of clinical pregnancy. The calculated observed/expected ratio (O:E) was 0.92 (95% confidence interval [CI]: 0.61–1.28). Eight studies reported on prediction of clinical pregnancy with a heartbeat, with a calculated O:E ratio of 0.77 (95% CI: 0.54–1.05). Four studies reported on live birth, with a calculated O:E ratio of 1.12 (95% CI: 0.26–2.37), and four studies reported on embryo ploidy status, with a calculated O:E ratio of 0.86 (95% CI: 0.42–1.27). Whilst the O:E ratios for clinical pregnancy, clinical pregnancy with a heartbeat, and embryo ploidy status outcome measures are promising and hold potential for the future, the findings from these 18 studies do not provide enough supporting evidence to suggest that AI-based models are better than current assessment tools in predicting outcomes.

"Given that the findings of this systematic review and meta-analysis are promising, but limited by the low number of studies included, further studies assessing the efficacy of AI-based models in predicting IVF outcomes are required."

Given that the findings of this systematic review and meta-analysis are promising, but limited by the low number of studies included, further studies assessing the efficacy of AI-based models in predicting IVF outcomes are required before considering their use as standard practice. ●

Testosterone Therapy Improves Pregnancy Outcomes in *In Vitro* Fertilisation

PRETREATMENT with testosterone therapy can increase likelihood of clinical pregnancy and live birth for poor responders undergoing *in vitro* fertilisation (IVF). The encouraging findings of a systematic review and meta-analysis of randomised controlled trials (RCT) were shared in a presentation at ESHRE 2022 on 5th July.

The literature review, conducted by researchers from Aristotle University of Thessaloniki, Greece, identified eight RCTs from between 2006 and 2021, which considered 760 individual cases. The studies described pretreatment with transdermal testosterone gel ahead of ovarian stimulation in IVF, with gel doses ranging 10.0–12.5 mg/day for pre-treatment durations of 10–56 days. Outcomes considered across the studies included clinical pregnancy; IVF therapy requirements, such as duration of ovarian stimulation and total dose of gonadotrophins; oestradiol levels; and IVF outcomes, including the number of cumulus–oocyte complexes retrieved and number of embryos transferred.

Pretreatment with testosterone gel associated with higher rates of clinical pregnancy (risk ratio: 2.25; 95% confidence interval: 1.54) and live birth (risk ratio: 2.07; 95% confidence interval: 1.09–3.92) in individuals classed as poor ovarian responders. The studies noted fewer days of ovarian stimulation therapy and a lower total dose of gonadotrophins required for those receiving testosterone pretreatment, as well as lower rates of cancellation because of poor ovarian response.

"Pretreatment with testosterone gel associated with higher rates of clinical pregnancy [...] and live birth."

The mechanism by which the testosterone pretreatment improved clinical outcomes in poor ovarian response in IVF relates to the role of androgens to increase the number of follicles at all stages, increase ovarian sensitivity to follicle stimulating hormone, and stimulate early-stage growth of follicles. Individual studies have considered the efficacy of testosterone pretreatment for improving clinical success of IVF in poor responders; however, this review and meta-analysis outlines clear conclusions for the success of this therapy. The review is limited by the total number of cases considered, as well as variability across RCTs regarding dosages and duration of pre-treatment, so further RCTs are indicated to confirm the conclusions and clarify best treatment practice. ●

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Interviews

EMJ team had the pleasure of interviewing two specialists: Mausumi Das, Consultant Gynaecologist and a Subspecialist in Reproductive Medicine, Hammersmith Hospital, Imperial College Healthcare NHS Trust, and Chelsea and Westminster Hospital NHS Foundation Trust, London, UK; and Signe Altmäe Department of Biochemistry and Molecular Biology, University of Granada, Spain, and Division of Obstetrics and Gynaecology, Karolina Institutet, Stockholm, Sweden

Featuring: Mausumi Das and Signe Altmäe

Mausumi Das

Consultant Gynaecologist and a Subspecialist in Reproductive Medicine, Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Hammersmith Imperial College Healthcare NHS Trust and Chelsea and Westminster Hospital NHS Foundation Trust, London, UK

01 What led you to pursue a career in reproductive medicine?

I decided to choose reproductive medicine as my career because of the amazing opportunities it would give me to make a fundamental difference in someone's life. For me, there can be no greater joy than helping men and women conceive and fulfil their dream of having a family. While working as research fellow in reproductive medicine at Queen Mary, University of London, UK, I thoroughly enjoyed learning about the clinical and scientific aspects of infertility and reproductive endocrinology. While doing laboratory-based research on polycystic ovary syndrome (PCOS) for my research doctorate, I was attracted to the fact that reproductive medicine offers excellent opportunities for translational research so that we can offer our patients pioneering treatments and state-of-the-art technologies. I found it fascinating that millions of babies have been born with the help of assisted conception techniques such as in vitro fertilisation (IVF) and intracytoplasmic sperm injection, and this simply would not have been possible without the development of IVF. I knew then that I wanted to pursue a career in reproductive medicine, and it has been an incredibly rewarding journey for me.

Reproductive medicine encompasses a wide range of specialties, including early pregnancy, reproductive endocrinology, PCOS, infertility, assisted conception, andrology, recurrent miscarriage, reproductive surgery, and management of the menopause. For me it has been a truly rewarding career choice and a privilege to be able to do what I love every day.

02 Your personal education and professional experience have involved you travelling to numerous destinations, such as Canada and the UK. Where do you believe you gained the most experience, and do you believe travelling was integral for you to make it to where you are today?

I completed my postgraduate specialisation in obstetrics and gynaecology in the UK, and was awarded a certificate of completion of training by the Royal College of Obstetricians and Gynaecologists (RCOG). During my obstetrics and gynaecology training in the UK, I gained wide ranging experience in various aspects of obstetrics and gynaecology. I subsequently undertook a 2-year RCOG accredited sub-speciality fellowship programme in reproductive medicine and surgery at the world-renowned McGill University in Montreal, Canada. As this was a busy, tertiary referral centre, I gained extensive experience and expertise in the management of subfertility, reproductive endocrinology disorders such as PCOS, and assisted conception. As it is a national referral centre for cancer patients seeking fertility preservation, I gained considerable experience in managing patients with cancer undergoing fertility preservation prior to gonadotoxic treatment, and this is an area of special interest and expertise for me. As the McGill Reproductive Centre is a pioneer in the technique of in vitro maturation of oocytes, I developed a special interest in this field, especially as it is aligned to the work that I had undertaken for my research doctorate on the mechanisms influencing folliculogenesis in PCOS.

I thoroughly enjoyed my fellowship programme in reproductive medicine at McGill University. Besides providing me with exceptional clinical experience and skills, the experience of working in a different healthcare system with people from different cultural backgrounds has helped me in my professional and personal development. I recently completed a Master of Public Health in Epidemiology from Harvard University, Cambridge, Massachusetts, USA, which provided me with advanced research and epidemiological skills that I can apply to address issues relating to patient care and population health research. I therefore truly believe that travelling abroad for my education and professional experience has helped me to expand my horizons, and has provided me with a unique set of experience and skills which has enabled me to provide the best possible care for my patients, and contributed significantly to my career as a reproductive medicine specialist.

03 You are currently the author of more than 30 peer-reviewed publications and book chapters in the field of reproductive medicine and infertility. What do you believe to be the current gaps in literature, and what topics merit greater attention?

The field of reproductive medicine has expanded rapidly, driven by the need to provide safe and effective treatment options for patients. Although these have led to new insights and discoveries, many fundamental

questions remain unanswered. There have been several advances in our understanding of the factors affecting embryo development, ranging from molecular and genetic mechanisms and the role of epigenetics to the effect of cancer and gonadotoxic agents. However, many aspects affecting embryo development remain unclear. Research is needed to identify optimal ovarian stimulation protocols in poor responders, and improve embryo culture and embryo selection techniques. Much of the available data is obtained from retrospective or small cross-sectional studies, limiting the validity of the findings. Well-designed, randomised studies will help to clarify these issues. Male reproductive health has often been neglected, and well-conducted studies to improve male fertility and identification of better methods of sperm selection for assisted conception are key research priorities. Moreover, there is a need for superior sperm cryostorage systems, including better systems for cryopreservation of testicular tissue. The application of precision medicine to improve clinical outcomes merits greater attention. Artificial intelligence systems based on deep learning algorithms and artificial neuron networks are being developed. These technologies may help to optimise sperm and embryo selection for assisted reproduction treatments, so that embryos with higher developmental potential can be selected and transferred. Furthermore, research is urgently required to provide enhanced fertility preservation treatment options for male and female cancer patients. Finally, large population studies are required to generate robust data on the impact of environmental factors on sperm and ovarian function and fertility outcomes.

04 What are the most significant changes you have seen in the field of reproductive medicine and gynaecology during your time working in the field?

The past decade has seen some truly remarkable breakthroughs in the field of reproductive medicine and gynaecology. Improved methods of oocyte cryopreservation have led to better success rates following

> **"For me, there can be no greater joy than helping men and women conceive and fulfil their dream of having a family."**

"The past decade has seen some truly remarkable breakthroughs in the field of reproductive medicine and gynaecology."

egg freezing and enhanced fertility preservation options for millions of women. Advances in embryo selection techniques have enabled reproductive medicine specialists to transfer a single embryo at a time, thereby reducing the chance of multiple pregnancy without compromising success rates. Ovarian stimulation protocols have been optimised to decrease the risk of ovarian hyperstimulation syndrome. Furthermore, progress has been made in optimising treatment regimens for older women and poor responders. Recent research has focused on providing better fertility preservation options for cancer patients about to undergo gonadotoxic therapies like chemotherapy and radiotherapy. Besides improved egg cryopreservation methods, there have been significant developments in ovarian tissue cryopreservation techniques along with in vitro maturation of oocytes. In an exciting development, doctors in Sweden reported in that a 36-year-old woman who had received a uterus from a 61-year-old friend, gave birth to a healthy baby in 2014. Although the procedure is still experimental, it offers hope to women without a uterus, or those who are not able to carry a pregnancy. In the field of male infertility, novel methods for selecting sperm for use in assisted conception have been introduced in recent years, although the optimum method for sperm selection is still unclear. Moreover, improvement in surgical sperm retrieval procedures such as microTESE have offered hope to thousands of men all over the world.

05 What are some points of emphasis you incorporate into practice to be the best consultant gynaecologist you can be?

Patients are at the heart of everything I do. I believe in a patient-centred approach, and involve patients in decision making about their care. I am curious and believe that medicine is a process of lifelong learning. I keep up to date and reflect on my practice regularly to ensure that I provide the best quality of clinical care to my patients. At the same time, I feel that it is important to be a good listener, to be compassionate and empathetic, and to have good communication and team working skills.

06 You are a member of several international learned societies, including the European Society of Human Reproduction and Embryology (ESHRE). How are the ESHRE and other societies using their position to educate surgeons, nurses, and trainees about the field of reproductive medicine?

International learned societies such as ESHRE and the American Society of Reproductive Medicine (ASRM) play a vital role in the advancement and practice of reproductive medicine. They achieve this by the sharing of information and knowledge, and by educating and training clinicians, nurses, embryologists, and reproductive medicine specialists of the future. They help to facilitate and disseminate research in human reproduction and embryology. They organise several educational courses throughout the year, and their e-learning platform is an excellent forum to keep abreast of the latest developments in the field.

The annual ESHRE conference is very well attended, and presents a great opportunity to attend state-of-the-art lectures, to network, and to exchange ideas.

07 Polycystic ovarian syndrome is one of the most common reproductive health diseases. Have you seen much improvement in its treatment over the last few years?

Although PCOS is one of the most common reproductive health disorders, there are still several challenges in its understanding and management. In recent years, greater emphasis has been laid on the long-term cardiovascular effects of PCOS and their management. Moreover, Type 2 diabetes and gestational diabetes are more prevalent in PCOS. Therefore, all women with PCOS should be screened for cardiovascular risk factors. Lifestyle intervention focusing on weight management is recommended first in women who are obese, as it improves general health and reduces adverse outcomes such as coronary heart disease and stroke. There is some evidence to show that metformin may also improve cardiometabolic health in women with PCOS. Lifestyle modification and metformin may improve endometrial dysfunction and pregnancy outcomes in women with PCOS. Although some studies have suggested that inositol therapy may be useful, large, well-designed, randomised trials to assess its efficacy are needed. It has been shown that carefully monitored ovulation induction protocols can achieve good cumulative pregnancy rates while minimising multiple pregnancy rates. Clomiphene citrate is still considered to be the first line treatment for ovulation induction. Letrozole is being increasingly used

an alternative to induce ovulation in patients who have not responded to clomiphene, and may improve clinical pregnancy rates and reduce time-to-pregnancy. Gonadotrophin ovulation induction is used as second line treatment. IVF with the gonadotrophin releasing (GnRH antagonist) protocol is now commonly used to minimise the risk of ovarian hyperstimulation syndrome. A multidisciplinary team approach may help patients to follow lifestyle interventions and improve their cardiometabolic and reproductive health.

"I believe in a patient-centred approach, and involve patients in decision making about their care."

08 How have you seen the advent of new technologies significantly impact the field of fertility preservation in recent years?

In recent years, there have been noteworthy advances in the field of fertility preservation, which have had a significant impact on the efficacy of fertility preservation options available to patients. Importantly, egg cryopreservation is no longer considered experimental. Improved oocyte cryopreservation techniques have led to vastly improved success rates following oocyte freezing. This has been especially important for women with cancer about to undergo gonadotoxic chemotherapy or radiotherapy. Furthermore, there have been major developments in ovarian tissue cryopreservation techniques, and research is ongoing to improve the safety and efficacy of this technology. Studies have also described novel methods of aspirating immature oocytes from the excised ovarian cortical tissue followed by in vitro maturation of the immature oocytes. Researchers have recently explored the feasibility of restoring fertility through spermatogonial stem cells in men with testicular failure. In the future, progress in stem cell research may help to restore fertility in cancer patients.

09 What advice would you give to someone hoping to pursue a career in reproductive health? Which lessons, if any, do you wish you had been taught as a young student?

For those wishing to pursue a career in reproductive health, I would say that it can be a very rewarding career choice. Besides being personally extremely satisfying, one can make a very positive and meaningful impact on the reproductive health and wellbeing of millions of men and women. Shadowing a reproductive medicine specialist for a few weeks is a great way of finding out what a typical day is like in the life of a reproductive medicine specialist, and therefore an excellent way to find out if it is the right career choice.

I would tell young students that life is a rollercoaster, filled with countless opportunities and novel experiences. Time management and being well organised are crucial. There will be difficulties along the way and being resilient is important. Take all advice onboard because it will help you succeed. Enjoying what you do is the key to success!

Signe Altmäe

Department of Biochemistry and Molecular Biology, University of Granada, Spain; Division of Obstetrics and Gynaecology, Karolina Institutet, Stockholm, Sweden

Q1 What led you to want to specialise in biomedicine (molecular biology) and particularly in reproductive health?

To be honest, I did not know what to study. I knew more clearly what I did not want to study, and biomedicine sounded interesting, although I did not know its full meaning. So, I chose this as it sounded more interesting than others. In my case, it has been a mixture of timing, coincidences, and luck. I've been always following the path that is exiting, challenging, and interesting for me. I think that, for me, the key point was meeting Andres Salumets and joining his group of reproductive biology and medicine in 2003.

Q2 What are your current research interests? Have these shifted since you began your career?

Yes, of course my interests are evolving, changing along with my experience and knowledge in the field. My current research interest is to understand what is the role of commensal microbes in the female and male reproductive tract, specifically to unravel the host-microbe interactions in reproductive functions. More and more evidence is demonstrating that reproductive sites that traditionally were considered sterile (e.g., uterus, testes) harbour commensal microbes on low levels, but we do not know what their role is. If they are there then must be function for them.

Q3 You read for two PhDs, the first at the University of Tartu, Estonia, and the second at the Karolinska Institutet, Stockholm, Sweden. What led you to read for a second doctorate, and how did these periods of focused study impact your research interests?

I started my PhD studies at the University of Tartu (in the group with Andres Salumets) and then I had an opportunity to do a 5-month research stay. I applied for funding from Estonia and research stay in Karolinska Institutet, Sweden and was granted both, so I could unite both fundings and finally perform a 10-month research stay instead. That was long enough time to realise that I would like to stay at the Karolinska Institutet and the only option to stay there was to apply for PhD funding. I was, again, lucky to get this funding and my 'path' was chosen; to conduct PhD studies at Karolinska Institutet under the supervision of Anneli Stavreus-Evers, but also to continue my research work with Salumets in Estonia. As I did not want to quit something that I had already started, I finalised my PhD studies in Estonia in parallel. Indeed, it was a period of focused study and work (a minimum 70 hours per week, I believe), but in those days that was my life; my lifestyle of 'living' in the laboratory, including weekends. It might sound sad, but I was very happy. In fact, I was with a bunch of international students, and we had a lot of fun together, working hard but also enjoying lunches, gym, saunas, and fikas together. The two key words for me from this intensive period are: enjoy and freedom.

Q4 You currently work at the University of Granada, Spain. How has the COVID-19 pandemic impacted your teaching and what have the effects been on your students? Have you found any unexpected benefits from the shift in remote teaching practices?

I have a researcher contract that includes rather small amount of teaching, so, of course, the pandemic has impacted my teaching (remote or in-person); but, altogether, it has been fine. The practical classes in the lab have been with a reduced number of students, about 50% of the class, which actually has been even nice. But what does bother me is that when some students greet me, I do not recognise them as I've never seen their faces. I also miss this student–teacher interactions that happen in the in-person classes (and classes without masks). In regard to research, the remote world has activated a lot of collaborations and, while I took part in international collaborations before, there are now days where I basically spend the whole day in online meetings, and this feels normal today.

Q5 You have written an impressive number of research papers and have also authored three book chapters to date. Can you pick out one or two that led you to discover something important, or of interest, in the field of reproductive health?

Every paper has a story behind it, but I believe we all remember our first 'baby', the first submission, and receiving comments and critics from reviewers, answering the reviewers and resubmitting, in short getting to know this world. But, if I were to highlight one work, then I must say it has been really exiting to enter into the transcriptome world (the analysis of RNA molecules) and to predict molecular interactions between the embryo and endometrium in order to understand the molecular dialogue, leading to embryo implantation in humans.

Q6 A paper that you co-authored entitled 'Genitourinary microbial screening for all infertile men?' was published in April 2022. What do you think are the benefits of genitourinary microbial screening and which conclusions did you come to in your research?

Genitourinary microbial screening would definitely provide answers (the confirmation or discarding) of possible microbial dysbiosis/imbalance that are otherwise not detectable and thereby could help to refine a diagnosis of infertility and treatment options. It feels like we are doing a puzzle, with every piece we get closer to the full picture; but there is still a lot to discover, starting from how we define what is 'normal' and 'abnormal'; what role the microbes in our body play, specifically in the upper reproductive tract; and what impact it has on oocyte fertilisation, embryo implantation, and pregnancy establishment.

> "It feels like we are doing a puzzle, with every piece we get closer to the full picture; but there is still a lot to discover."

Q7 You have received many prestigious awards from all over Europe, including the Young Scientist Award from the Egon and Ann Diczfalusy Foundation for Supporting Research in Reproductive Health. Which of your awards are you most proud of and how do you feel that this recognition has helped your career to develop?

I don't know how other researchers feel but, at least for me, all the awards have been important to my recognition, independently of the prestige. I have been equally happy in receiving European Society of Human Reproduction and Embryology (ESHRE) award for the best poster as receiving a local distinction; both have made me equally happy and proud. In the research field, we usually get (constructive) critiques (reviewers commenting manuscripts, research projects, etc.) and the positive feedback is lacking. However, I am truly grateful for all the prizes and awards. I believe that the recognition has, in the end, helped me to get more recognition and also funding, which are crucial for any scientific career.

Q8 How has the landscape of reproductive health shifted during your career? In your opinion, which breakthroughs have had the biggest impact on current practices?

Just the other day I was saying that I feel that there has been a dramatic shift in the field of reproductive health. When I started 20 years ago, molecular studies involved analyses gene by gene: the single gene approach. Then, 13–14 years ago, the 'omics' revolution arrived in the field of reproductive health and today we are able to provide molecular tests for personalised infertility treatment protocols. Now, we are mainly exploiting transcriptome data; however, there is still much to see of the microbiome, metabolome, and proteome world.

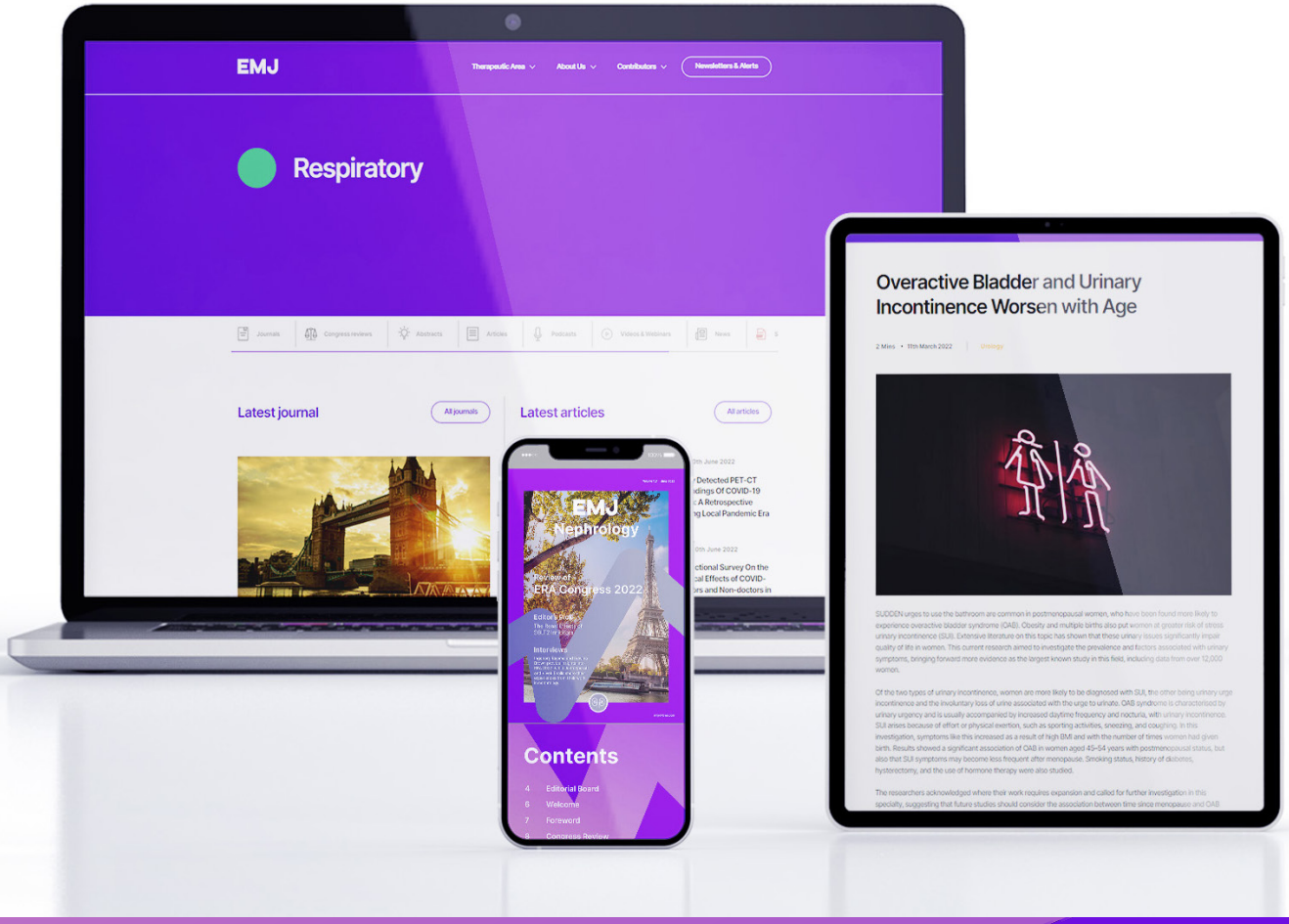
Q9 Which new technologies and techniques in the field of reproductive health are you most excited about and why? How do you feel these innovations will impact healthcare in the future?

I'm excited about the microbiome testing, the unexplored options that the detection of 'good' bacteria and 'bad' bacteria (the eubiotic and dysbiotic) microbial sites offer. We still need to clarify what is 'normal' in microbial composition in the upper reproductive tract (both female and male) and how it can change as a result of disease, whether it is a cause of consequence. But once we have this knowledge, we could aim to modify, to improve the dysbiotic conditions (with nutrition and local pro- and prebiotics) and, hopefully, we could cure some conditions or even prevent different diseases.

Q10 What advice would you give to someone hoping to pursue a career in reproductive health? Which lessons, if any, do you wish you had been taught as a young student?

I don't remember any specific lessons, but I truly believe (and it applies to any field in any profession) that you have to like what you do. And don't worry if you don't know what you want today; start eliminating options that you are sure you don't want and, in the end, there will be fewer 'paths' to choose. Also, it is normal to make mistakes, and a problem is not a problem if it is fixable. And to always try to give your best.

When entering the research field, it is important that you do research with people you understand and enjoy being around.



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The Value of Serum Follicle-Stimulating Hormone in Predicting Successful Surgical Sperm Retrieval in Cases of Male Infertility: A Literature Review

Editor's Pick

My pick for this issue is the article entitled: 'The Value of Serum Follicle-Stimulating Hormone in Predicting Successful Surgical Retrieval in Cases of Male Infertility: A Literature Review', which faces the important problem of predicting the attainment of spermatozoa after testis biopsy in patients with azoospermia. Several papers in the literature analysed the predicting ability of serum follicle-stimulating hormone (FSH) levels in sperm retrieval for testicular sperm extraction. Here, the authors reviewed 35 articles with the aim of identifying a possible threshold FSH value. In general, successful sperm retrieval is associated with lower FSH levels (>8.5). Whether successful sperm retrieval is associated with live birth is, however, not clear from these studies. Clearly, as suggested, more studies will be necessary, particularly those considering other testicular serum markers as additional predictors.

Elisabetta Baldi
University of Florence, Italy

Authors: *Meera Ramcharn,[1] Tom Hampshire,[2] Oleg Tatarov,[1] Shahana Abdu,[1] Paul Knaggs,[1] Arianna D'Angelo[1]

1. Wales Fertility Institute, Swansea Bay University Health Board, Cardiff, Wales, UK
2. Centre for Medical Education, Cardiff University, Wales, UK

*Correspondence to meera.ramcharn@wales.nhs.uk

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Abstract

Azoospermia is a common cause of male infertility; however, surgical sperm retrieval (SSR) and subsequent intracytoplasmic sperm injection offers couples the chance to have a biological child. SSR success is highly variable and dependent on a number of factors. One such factor is male follicle-stimulating hormone (FSH), which has been researched extensively. The aim of this literature review is to ascertain if there is a 'cut off' FSH value that correlates with successful SSR, whether this value differs depending on method of SSR, and if there is a correlation between male FSH level and obstetric outcomes. Thirty-five articles were identified and reviewed, with 10 papers suggesting FSH cut off values. These ranged from <8.5 to <25.0 IU/L, with a

mean value of 14.0 IU/L. Generally the results suggested that lower FSH values were associated with increased SSR success. Few papers considered pregnancy and birth outcomes following intracytoplasmic sperm injection with surgically retrieved sperm, and there was no clear correlation with male FSH levels. Clinical implications include considering FSH results when counselling patients about both SSR and intracytoplasmic sperm injection. Suggested future research implications are to further investigate the predictive role of FSH in combination with other clinical and endocrinological markers.

Key Points

1. Surgical sperm retrieval is one method to provide males with azoospermia the chance to father a biological child but success depends on numerous variables, including follicle-stimulating hormone.
2. Lower follicle-stimulating hormone values were associated with increased surgical sperm retrieval success, especially in obstructive azoospermia.
3. Infertility is associated with feelings of disappointment and a loss of control worldwide, and clinicians have an ethical obligation to provide evidence-based management and individualised care.

INTRODUCTION

Male factor aetiologies account for approximately 50% of infertility in couples,[1] with azoospermia diagnosed in up to 15% of infertile males.[2] The World Health Organization (WHO) semen analysis parameters are universally used and define azoospermia as an absence of spermatozoa identified in wet or centrifuged ejaculate samples.[3] Azoospermia is classified into either obstructive (OA) or non-obstructive causes (NOA), with impaired spermatogenesis. Treatment for both consist of surgical sperm retrieval (SSR) with a variety of techniques. These include testicular sperm extraction with microscopy with (mTESE) or without (TESE), testicular sperm aspiration, microsurgical epididymal sperm aspiration, percutaneous epididymal sperm aspiration (PESA), and fine needle aspiration (FNA). Surgically retrieved spermatozoa are then utilised in intracytoplasmic sperm injection (ICSI). This allows males with azoospermia to father genetically-related children, and remove the necessity for sperm donors or adoption.

Despite several methods, SSR outcomes can be of variable success, particularly in cases of NOA.[4] Spermatogenesis is controlled by a complex neuroendocrine axis including follicle-stimulating hormone (FSH). Although the relationship between FSH and SSR has been explored previously, the results have not always been consistent. Therefore, the current evidence was assessed through a literature review to ascertain if FSH levels can correlate with SSR outcomes, in order to update clinicians and help patients make a more informed choice.

METHOD

Objectives

To evaluate if pre-operative serum FSH levels are predictive of subsequent successful SSR (TESE, mTESE, testicular sperm aspiration, microsurgical epididymal sperm aspiration, PESA, FNA) in cases of azoospermia prior to ICSI.

Outcomes

The primary outcome is to ascertain if FSH is predictive of successful SSR, and to determine a 'cut off' value. Secondary outcomes include whether different SSR methods have different cut off values, and if there is a correlation between FSH and clinical pregnancy or live birth outcomes following ICSI.

A literature review was conducted by performing a Medline search via Ovid from January 2002–2021. The search terms 'FSH', 'follicle-stimulating hormone', 'surgical sperm retrieval', 'testicular sperm extraction', 'microdissection testicular sperm extraction', 'testicular sperm aspiration',

Figure 1: Flowchart summarising the screening process of article selection.

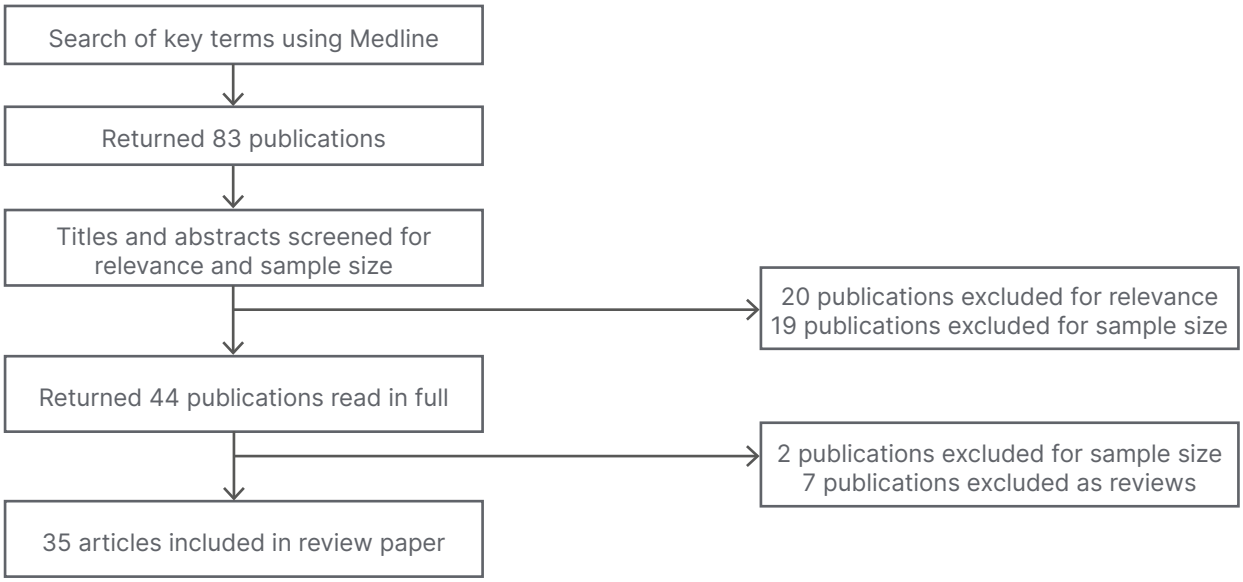

Table 1: Study characteristics and association between follicle-stimulating hormone and surgical sperm retrieval.

	Study Design	Sample size	Azoospermia classification	SSR technique	Results	Suggested FSH 'cut off' value	Comments
Amjad et al.[5]	Prospective cohort study; single centre	100	Mixed	TESE/mTESE	Mean FSH significantly higher in unsuccessful group (14.12±2.30 versus 23.87±2.34; p=0.004; AUC: 0.675 (p<0.05)	12.16 µIU/mL; sensitivity: 67.0%; specificity: 62.0%	Pregnancy outcomes not considered
Majzoub et al.[6]	Retrospective analysis; single centre	297	NOA	TESA±mTESE	Median FSH successful versus unsuccessful SSR 5 (2.0-9.5) IU/L versus 12 (5.0-20.0) IU/L; (p=0.000); AUC: 0.742	<8.5 IU/L (95% CI: 0.13–0.04)	Pregnancy outcomes not considered
Cayan et al.[7]	Retrospective observational analysis; multicentre	327	Mixed	mTESE	Group with FSH <17.25 µIU/mL significantly more likely to have successful SSR (72.3% versus 44.4%; p=0.000)	N/A	Pregnancy outcomes considered, but not in regard to FSH; all had history of cryptorchidism
Liu et al.[8]	Retrospective analysis; multicentre	294	NOA	TESA/mTESE	Successful TESA FSH (12.20±5.72 versus 19.60±8.68; p=0.003);	N/A	Pregnancy outcomes not considered

Table 1 (continued):

					successful mTESE FSH (31.62±13.76 versus 25.51±12.06; p=0.001); FSH cut off for TESE <19 µIU/mL and >19 µIU/mL for mTESE (ROC analysis)		Excluded patients with ejaculatory disorders or hypogonadotropic hypogonadism; defined success as retrieval of one sperm
Zeadna et al.[9]	Retrospective cohort study; single centre	119	NOA	TESE	No statistical difference in FSH between successful and unsuccessful groups; FSH variable in gradient-boosting trees predictive model (AUC: 0.807; FSH cut-off 18.95 IU/L; variable importance: 0.014)	N/A	Pregnancy outcomes not considered; excluded patients with gonadotoxin usage and hypogonadotropic hypogonadism
Zhang et al.[10]	Retrospective observational analysis; single centre	155	NOA	mTESE	FSH >24.8 IU/L significantly higher SSR success than where FSH >12.4 IU/L (p=0.033)	N/A	Pregnancy outcomes not considered; only idiopathic NOA included
Jahromi et al.[11]	Prospective cross-sectional study; single centre	171	NOA	mTESE	N/A	14.6 µIU/mL; AUC: 0.88 (95% CI); sensitivity: 83.5% (73.5–90.9%); specificity: 80.3% (69.5–88.5%)	Pregnancy outcomes not considered; excluded patients with hypogonadotropic hypogonadism
Nariyoshi et al.[12]	Retrospective clinical audit; two centres	806	NOA	mTESE	FSH <14 IU/L; AUC 0.61	N/A	Pregnancy outcomes not considered
Barbotin et al.[13]	Retrospective analysis; single centre	225	NOA	TESE	FSH statistically lower in successful group (median: 19.2 IU/L versus 23.6 IU/L; p=0.007)	N/A	Pregnancy outcomes considered, but not with respect to FSH; exclusively patients with cryptorchidism; excluded concurrent aetiologies for azoospermia
Busch et al.[14]	Retrospective analysis; single centre	1,075	Mixed	mTESE	FSH significant in success in unexplained azoospermia (p=0.006)	N/A	Pregnancy outcomes not considered; extensive exclusion criteria
Ma et al.[15]	Retrospective cohort analysis; multicentre	597	NOA	FNA	Increased FSH significantly associated with failed SSR (p<0.001)	N/A	Pregnancy outcomes not considered

Table 1 (continued):

Blok et al.[16]	Retrospective analysis; single centre	231	OA	OESA	Univariate analysis FSH: associated with SSR success rates (p=0.01); multivariate logistic regression: FSH negatively associated with SSR success (p=0.021; OR: 0.87; 95% CI: 0.78–0.98)	N/A	Pregnancy outcomes considered, but not with respect to FSH
Zhu et al.[17]	Prospective analysis; single centre	403	NOA	TESE/ mTESE	FSH successful SSR: AUC 0.87 (95% CI: 0.83–0.90). If FSH >12.4 IU/L: ROC AUC: 0.55 (95% CI: 0.48–0.63); suggested cut-off ≤15.45 IU/L (sensitivity: 36.67%; specificity: 78.17%)	≤9.00 IU/L; sensitivity: 80.75%; specificity: 90.00%	Pregnancy outcomes not considered; success defined as retrieval of spermatozoa or spermatids; no reference range for elevated FSH in analysis
Amer et al.[18]	Retrospective cross-sectional analysis; single centre	1,395	NOA	mTESE	No significant difference in mean FSH in successful and unsuccessful groups: (19.52±13.08 µIU/mL versus 19.81±14.21 µIU/mL; p=0.767); ajority in successful group had 'high FSH': >8 IU/L (p=0.02); logistic regression analysis: 'high FSH' significantly associated with success (OR: 1.6; 95% CI: 1.2–2.1; p=0.003)	N/A	Pregnancy outcomes not considered; extensive exclusion criteria
Maglia et al.[19]	Cross-sectional study; single centre	145	NOA	TESE/ mTESE	mTESE significantly more successful than TESE if FSH >18 µIU/ml (60.0% versus 12.9%; p=0.001) Overall cohort analysis: FSH independent predictor of SSR failure (OR: 1.11; p=0.008)	N/A	Pregnancy outcomes not considered; males in interracial couples excluded
Gnessi et al.[20]	Retrospective analysis; single centre	486	Mainly NOA	TESE	Successful SSR correlated with lower FSH (15.70±12.22 versus 22.51±12.11; p< 0.01; OR: 0.96 /µIU); FSH included in SSR prediction score: AUC 95% CI: 0.843	N/A	Pregnancy outcomes not considered; all had previously failed SSR; 56/486 males had severe oligospermia or necrozoospermia
Caroppo et al.[21]	Retrospective analysis; single centre	356	NOA	TESE	Mean FSH significantly lower in successful TESE group (16.1, [95% CI: 1.79] versus 22.4 [95% CI: 2.32]; p<0.0001)	N/A	Pregnancy outcomes not considered; success defined as number of viable sperm equalled number of collected oocytes

Table 1 (continued):

Salehi et al.[22]	Retrospective cohort study; multicentre	170	NOA	TESE	Higher FSH correlated with lower chance of sperm retrieval (p<0.01)	N/A	Pregnancy outcomes not considered; success defined as ≥1 spermatozoa (regardless of motility)
Cissen et al.[23]	Retrospective cohort study; nationwide multicentre	1,371	NOA	TESE	FSH significantly lower in successful group (OR: 0.98; p=0.003; AUC: 0.64)	N/A	Pregnancy outcomes not considered
Guler et al.[24]	Retrospective analysis; single centre	271	NOA	TESE	Decreased FSH levels (11.0 9.5 IU-1 versus 22.3±16.0 IU-1) correlated to successful SSR (p=0.000)	N/A	Pregnancy and live birth rated considered, with no significant difference (p=0.817 and p=0.228, respectively); FSH best predictor of a successful TESE
Cetinkaya et al.[25]	Retrospective analysis; single centre	191	NOA	mTESE	FSH significantly higher in failed SSR group (24.9±15.2 versus 17.5±14.1; p=0.001) Idiopathic NOA showed FSH as an independent predictive factor for SSR outcome	15 µIU/ml; sensitivity: 75%; specificity: 51.2%; p=0.001; AUC: 0.656	Pregnancy outcomes not considered
Ramasamy et al.[26]	Retrospective review; single centre	1,026	NOA	mTESE	FSH not significant (p=0.66)	N/A	Pregnancy outcomes not considered
Abdel Raheem et al.[27]	Retrospective analysis; single centre	388	Mixed	TESE/ mTESE	NOA: increased FSH strongly negatively correlated to SSR rate (r=-0.208; p=0.001)	N/A	Pregnancy outcomes not considered; 112 patients had OA, all with normal FSH levels; 276 patients had NOA; 56% had raised FSH; success reported per testes
Enatsu et al.[28]	Retrospective analysis; single centre	329	NOA	mTESE	No FSH difference (p=0.42)	N/A	Pregnancy outcomes not considered; excluded males with normal FSH, testicular volume, or hypogonadotropic hypogodanism
Huang et al.[29]	Prospective analysis; single centre	305	NOA	TESE	FSH significantly lower in successful SSR (p<0.001)	11.05 µIU/mL; sensitivity: 83.5%; specificity: 74.5%	Pregnancy outcomes not considered; excluded patients with testosterone or gonadotrophin therapy

Table 1 (continued):

Nowroozi et al.[30]	Controlled cross-sectional study; single centre	385	NOA	TESA/ TESE	Mean FSH significantly lower in successful TESA group 13.0±4.7 IU/L versus 23.2±6.1 IU/L (p<0.001) FSH <15 IU/L was predictive of successful SSR with TESA (OR: 4.8; p=0.001)	N/A	Pregnancy outcomes not considered
Boitrelle et al.[31]	Retropective case series; single centre	280	NOA	TESE	FSH statistically lower in successful group (p=0.003; AUC: 0.656)	<20.5 IU/L; sensitivity: 68.5; specificity: 55.7; PPV: 63.8; NPV: 60.8	Pregnancy and live birth rates considered; excluded males with hypogonadotropic hypogonadism
Ramasamy et al.[32]	Retrospective analysis; single centre	126	NOA	Repeat mTESE	FSH significantly lower in successful group (23.1±12.4 versus 29.2±12.8; p=0.04)	N/A	Pregnancy outcomes considered, but not in respect to FSH
Ma et al.[33]	Retrospective analysis; single centre	280	NOA	TESE	FSH significanly lower in successful group in training set (13.7 ± 6.8 IU/L versus 16.2 ± 5.8 IU/L; p=0.02). Difference not significant in testing set (p=0.09)	14.32 µIU/L; sensitivity: 70.7%; specificity: 68.2%	Pregnancy outcomes not considered
Tuttleman et al.[34]	Retrospective analysis; single centre	283	Mixed	TESE	Lower FSH associated with higher chance of success. ROC analysis (AUC: 0.71; p<0.0001)	10 U/l (95th percentile; n=179)	Pregnancy outcomes not considered; excluded males with oncological aetiologies
Zitzmann et al.[35]	Retrospective cohort study; single centre	203	Mixed	TESE	FSH significantly lower in: successful SSR 4.8 IU/L (1.4–40.0) versus 17 IU/L (1.2–47.8; p<0.001); achieving clinical pregnancies 4.5 IU/L (range: 1.4–19.3) versus 6.6 IU/L (range: 1.2–47.8; p=0.009); live births 4.4 IU/L (range: 2.1–19.3) versus 10.9 IU/L (range: 1.2–47.8; p=0.014). FSH >20 IU/L; ROC: 100% specificity for prediction of no pregnancy (p=0.008) or no live birth (p=0.013)	N/A	Pregnancy and live birth rates considered; success defined as elongated spermatids extracted; male cigarette smokers included
Samli et al.[36]	Retrospective analysis; single centre	303	NOA	TESE	No significant difference in FSH SSR rates (p=0.35)	N/A	Pregnancy outcomes not considered

Table 1 (continued):

Raman et al.[37]	Retrospective analysis; single centre	275 males; 321 TESE	NOA	TESE	No significant difference in FSH SSR rates in patients with cryptorchidism (p=0.22) or without (p=0.53)	N/A	Pregnancy outcomes considered but not in respect to FSH 38/275 had cryptorchidism
Friedler et al.[38]	Retrospective analysis; single centre	175	Mixed	PESA/ TESE	No significant correlation with FSH and successful SSR, implantation rate, pregnancy or miscarriage	N/A	Pregnancy outcomes considered
Vernaeve et al.[39]	Prospective analysis; single centre	185	NOA	TESE	N/A	<25 IU/L; sensitivity: 74.3%; specificity: 44.3%; AUC: 0.56	Pregnancy outcomes not considered; patients with hypogonadotropic hypogonadism excluded

AUC: area under receiver operating characteristic curve; CI: confidence interval; FSH: follicle-stimulating hormone; FNA: fine needle aspiration; mTESE: testicular sperm extraction with microscopy; N/A: not applicable; NOA: azoospermia with a non-obstructive cause; NPV: negative predictive value; OA: azoospermia with an obstructive cause; OESA: open epididymal spermatozoa aspiration; OR: odds ratio; PESA: percutaneous epididymal sperm aspiration; PPV: positive predictive value; ROC: receiver operating characteristic curve; SSR: surgical sperm retrieval; TESE: testicular sperm extraction without microscopy.

'percutaneous epididymal sperm aspiration', 'azoospermia*', and 'predict*' were used. Eighty-three articles were identified and screened by the title and abstracts. Inclusion criteria applied to the studies were English language papers, randomised controlled trials (RCT), cohort studies, case-control studies, retrospective and prospective studies, and human-only studies. Exclusion criteria included papers with sample sizes of n=<100, letters to the editor, rapid response articles, case reports, review articles, and abstract proceedings. Two of the authors screened the results and reviewed the 35 full text papers included in the final analysis. Figure 1 depicts the screening processing.

RESULTS

Thirty-five articles met the inclusion criteria and were analysed. Table 1 outlines the study design, SSR technique, results, and authors' comments about the selected articles.

Twenty-seven studies considered NOA only; one study considered OA only; and seven studies included mixed aetiologies for azoospermia. Ten out of the 35 studies gave cut off FSH values predicting SSR success. These ranged from <8.5 to <25.0 IU/L, with a mean value of 14.0 IU/L. The results suggest lower FSH levels are associated with increased SSR success, although five papers did not demonstrate any significant relationship between FSH levels and SSR outcomes. Nine publications described pregnancy or birth related outcomes in addition to SSR results.[7,13,16,24,31,32,35,37,38] Five of these papers described ICSI outcomes following SSR procedures and consideration to FSH levels.

DISCUSSION

Indications for Surgical Sperm Retrieval

NOA is more common than OA, which is reflected in the respective proportion of articles covering each diagnosis. Additionally, some cases of OA can be treated with surgical correction to restore normal anatomy and avoid the necessity for SSR. However, males may show a continuum of disorder, with features of both NOA and OA.[7,35] Therefore, classification of

azoospermia may be of debatable value when counselling couples regarding treatment options, and an individualised approach to each case is preferred.

NOA testicular histopathological classifications include normal spermatogenesis, hypospermatogenesis, maturation arrest, and Sertoli cell-only syndrome. Genetic anomalies such as Klinefelter syndrome and Y chromosome microdeletions were in the exclusion criteria of 10 of the papers,[5,15,17-19,21,29,30,34,39] despite being well-established causes of azoospermia. Huang et al.[29] did identify FSH was not predictive for males with Klinefelter syndrome. Papers had variable exclusion criteria to decrease the risk of confounders; however, this made comparison by the authors more challenging.

Surgical Sperm Retrieval Techniques

Some publications compared mTESE with traditional techniques. Majzoub et al.[6] found that although FSH levels <8.5m IU/mL gave similar success rates between the two techniques, when all FSH ranges were considered, mTESE was more successful.

The authors' results have previously stated that SSR is less successful when males have higher pre-operative FSH levels, yet these articles suggest that males with higher FSH levels may benefit from microdissection techniques.[8,19,34] However, the number of publications comparing the two methodologies were very limited. Microdissection SSR requires advanced surgical training and is a longer procedure, therefore conferring marked resource implications and may reduce cost effectiveness.

FNA and PESA are simple, low cost, well-tolerated procedures, but as they are performed blind, they can yield less sperm compared to other SSR methods. Males may then have to undergo an additional TESE. Only one article about FNA[15] and one article including PESA[38] was identified, which is insufficient to conclude what FSH values would confer benefit from these procedures.

Strength of Evidence

The main limitations of these results are the heterogenous design of studies, and quality of evidence yielded. No RCTs were identified; however, it may not be feasible to apply RCT methodology to this topic, especially in blinding for surgical procedures.

Only five of the studies were prospective.[5,11,17,29,39] All of these papers are single-centre studies only; however, each identifies a FSH cut off for successful SSR, albeit these are still varied: <9 to <25 IU/L. Future prospective studies may help to refine the cut off range. For the remaining papers, it is well established that retrospective studies can carry bias when collecting data, or that crucial data may be missing that can affect reporting.

Many of the studies utilised regression analysis to form a predictive tool to calculate SSR success. Some of these studies included FSH in their algorithms, as they found it to be a significantly predictive factor.[23,25]

Alternative Variables for Surgical Sperm Retrieval Success

Frequently described clinical markers in the analysed papers included age and BMI. Testicular volume was considered most commonly,[6,8,10,15,31-34] but with varying ranges, again making a subgroup analysis difficult. Biomarkers include testosterone,[21] fructose,[34] α-glucosidase[34] and inhibin B,[29,39] which was described most frequently, and with good diagnostic accuracy.[17,31] The scope of this review intentionally did not consider the role of other variables as FSH is a cheap, established, readily available test. The authors therefore chose to focus on the potential diagnostic benefits of this sole marker.

Limitations

All SSR methods were considered in this review, and there may be different FSH cut offs depending on technique.[8] Although many of the studies described surgical techniques and gamete processing, variation is likely to be high. This mirrors global clinical practice, and so may not be a significant limitation to the results.

As previously highlighted, the vast majority of articles regarded NOA, which can bias the inferred application of FSH from this review. When the seven papers which included OA and mixed aetiologies are analysed separately, they are consistent with the existing literature:

males with OA have lower FSH and far higher successful SSR compared to males with NOA and higher FSH levels.[5,27,38] Little could be concluded from the remaining papers, as they either did not stratify FSH levels or SSR outcomes according to azoospermia classification.

The studies have varying criteria for successful SSR.[9,21] For example, one study[8] regarded success as retrieval of one sperm, which may be less clinically relevant for ICSI. Different studies used varying normal FSH ranges,[34,36] and so descriptions of 'high' or 'low' levels should be interpreted with caution.

Pregnancy and Birth Outcomes

The ultimate aim for SSR is to produce a healthy baby when combined with ICSI, so it is interesting that so few publications have considered these outcomes. Song et al.'s[40] research used FSH as part of a multivariable model, finding FSH significant in prediction of obtaining clinical pregnancy. This study was excluded from this review, as SSR had already occurred and was not the focus of the paper. Zitzmann et al.[35] identified 100% specificity for predicting no pregnancy or live births with FSH >20 IU/L (p=0.008). Meanwhile, alternative authors did not find FSH significant in predicting such outcomes.[24,31,38] Therefore the correlation between preoperative FSH in SSR, ICSI, and obstetric outcomes seems contested, and warrants further investigation.

Clinical Implication

It is well established that infertility is associated with feelings of disappointment and loss of control, independent of ethnicity and culture.[41] The included studies reflect the global scale and impact of azoospermia and SSR treatment. In addition to marked psychological burden, SSR confers similar risks to any surgical procedure: bleeding, infection, scarring, and associated hypogonadism,[42] which may further decrease spermatogenesis for repeat procedures.

Clinicians therefore have an ethical obligation when offering fertility treatment to couples, and not adopt a 'one size fits all' approach. If ICSI is likely to be futile for specific couples,[35] clinicians also have a duty to female partners as oocyte collection carries the previously stated risks in addition to ovarian hyperstimulation syndrome, which can be fatal.

Additional Authors' Perspective

This work differs from previous reviews, which have either solely focused on microsurgical techniques,[43] or the role of FSH in NOA SSR only.[44]

Additionally, this review summarises important inclusion and exclusion criteria, which may be utilised when providing evidence-based care to specific subsets of patients. Conversely the authors chose to cover all forms of SSR and azoospermia for generalisability of results for patients and clinicians, or where microsurgery may not be available.

CONCLUSION

The authors performed this review with an aim to determine if FSH values can be correlated with success of SSR and, if so, to define a cut off value. This will allow clinicians to counsel patients whether SSR with ICSI is likely to be efficacious, or if pursuing non-biological methods to achieve parenthood may be more appropriate. This review demonstrates that there is an association between lower serum FSH levels and increasingly successful SSR. The authors have not identified a discriminative FSH result to accurately predict SSR outcomes, although a suggested range of values were described. To further elucidate this link, the authors suggest future work should continue to focus on FSH used in combination with other endocrinological and clinical markers.

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Progesterone Resistance in Endometriosis

Authors:	Jeevitha Poorasamy, Jayasree Sengupta, Asmita Patil, *Debabrata Ghosh Department of Physiology, All India Institute of Medical Sciences, New Delhi, India *Correspondence to debabrata.ghosh1@gmail.com

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Abstract

Endometriosis is characterised by the presence of endometrium-like tissue on the pelvis and other organs. Progesterone resistance due to suppressed progesterone receptor (PGR) expression and action is a general feature of endometriosis and is a cause of endometriosis-associated chronic pelvic pain, infertility, inflammatory disorders, and cancer. It appears that progesterone receptor polymorphisms may not be associated with the susceptibility to endometriosis. On the other hand, PGR expression and activity in target cells is significantly dysregulated in both eutopic and ectopic tissues compared with control endometrium. However, the underlying epigenetic mechanisms for PGR suppression in the eutopic tissue are different from ectopic tissue. The aim of this paper was to present an overview of different aspects of progesterone resistance and its application in endometriosis. Finally, this article also presents a few important, unmet questions related to the failure of progesterone treatment in alleviating clinical conditions in endometriosis.

Key Points

1. Endometriosis affects nearly 10% of females of reproductive age; progestin treatment fails in a large number of these patients, likely due to progesterone resistance.

2. Progesterone resistance may be due to suppressed progesterone receptor expression and action and is a cause of endometriosis-associated chronic pelvic pain, infertility, inflammatory disorders, and cancer.

3. There is an urgent need for deeper understanding of the cellular and molecular mechanisms of progesterone resistance in endometriosis, for both ectopic and eutopic tissues, to underpin novel approaches to treatment.

INTRODUCTION

Growth of endometrial tissue outside the uterus, frequently but not exclusively, in the pelvic structures gives rise to endometriotic lesions in the peritoneum (peritoneal endometriosis), the ovary (ovarian endometriosis or endometrioma), and the deep pelvis (deep infiltrating endometriosis), and infrequently in the distant organs.[1] According to Sampson's theory, deposits of viable endometrial cells following their reflux into the peritoneal space via the fallopian tubes during menstruation may adhere and grow, and give rise to endometriosis.[2] In an elegant review, Redwine[3] challenged this theory and demonstrated, by analysing a large number of parameters, that endometriosis tissue is primarily reflected dissimilarity than similarity with eutopic endometrium in the uterus, including inadequate secretory differentiation in endometriotic cells under progesterone dominance during the luteal phase.

Nisolle and Donnez[4] speculated that inadequate secretory maturation in the endometriosis might cause from the reduction in progesterone receptor (PGR). Zeitoun et al.[5] and Attia et al.[6] observed that 17-β-hydroxysteroid dehydrogenase type 2 (17β-HSD2), the activity of which transforms oestradiol to less potent oestrogen (oestrone) and is stimulated by progesterone in endometrial glands, was significantly reduced in endometriotic tissue during the luteal phase, along with markedly repressed levels of immunoprecipitable PGR throughout the menstrual cycle.

The fact that progestin treatment fails to regress endometriosis in three out of 10 females is also indicative of inadequate machinery of progesterone action in the endometriotic tissue.[7,8] These observations were suggestive of the absence of certain responses to progesterone action.

Taking these observations together, a theory of 'progesterone resistance' as the mediator of pathogenesis of endometriosis was forwarded in the 2000s. Since then, though more intensely in the last decade, the theory has been under scrutiny.[8-12] An overview of this theory and its application in explaining the pathogenesis of endometriosis and its management will be presented in this paper.

PROGESTERONE RECEPTOR

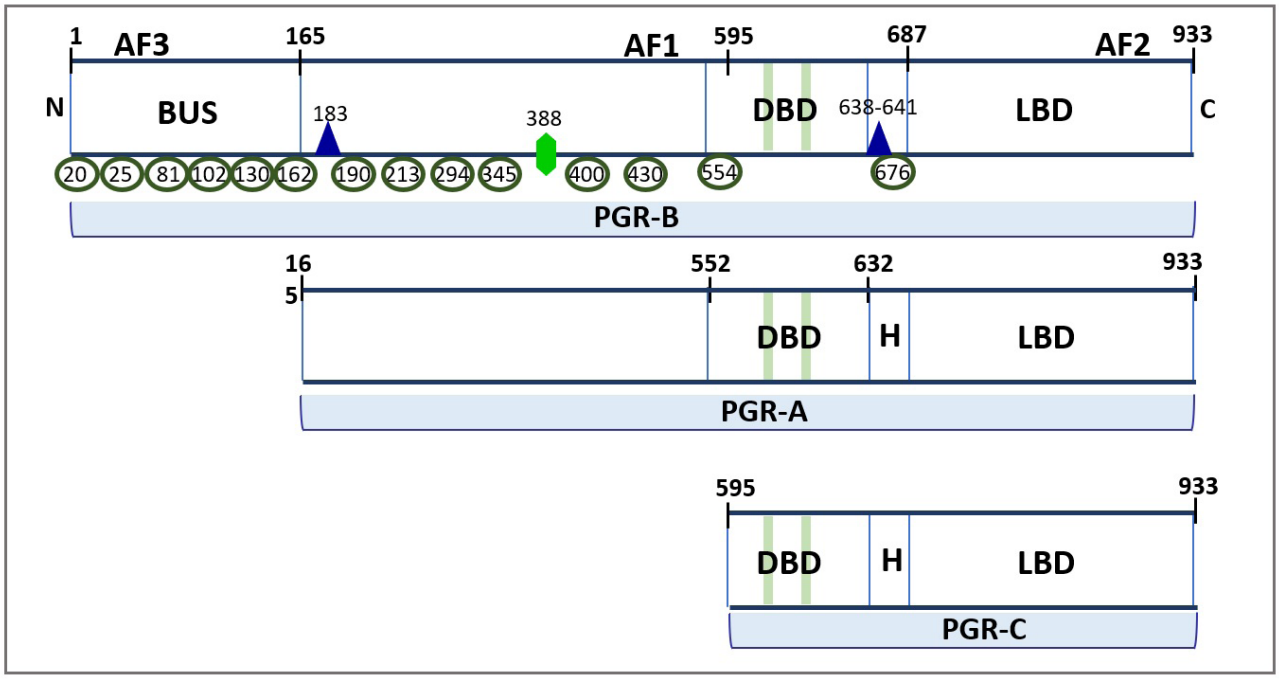
Progesterone is a steroid hormone primarily synthesised by the ovaries and adrenal glands, and also by the placenta during pregnancy. Progesterone, although quintessentially the 'pregnancy hormone', also plays an important role in several non-reproductive tissues such as the breast, heart and vascular system, brain, and bones.[13] The physiological actions of progesterone in target cells are mediated by its binding to PGRs: classical and non-classical. Typically, classical PGR regulates the expression of progesterone responding genes, which result in slowly emerging, long lasting cellular responses. On the other hand, progesterone binding to non-classical PGR activates secondary messengers and signal transduction pathways and mediates rapid responses. A detailed discussion on the physiology of different types of PGR is beyond the scope of the present paper; however, the authors present a synopsis on the topic in the following section. Interested readers may be referred to the comprehensive review articles that covered different aspects of PGR in mammalian cells and specifically in endometrium.[11,14-19]

Classical Progesterone Receptors

There are two main isoforms of classical PGR: PGR-A (94 kDa) and PGR-B (120 kDa). Both are transcribed from the same gene, but by two different promoters. As schematically presented in Figures 1 and 2, these two isoforms are very similar except that PGR-A lacks 164 amino acids that are present at the N-terminus of PGR-B. Unbound PGR in cytoplasm are complexed with chaperone proteins.[14-19] Several lines of evidence suggests that PGR-A is functionally distinct from PGR-B, and thus tissue-specific distribution patterns of PGR-A and PGR-B result in the observed diversity of progesterone-mediated actions.

Generally, PGR-B is the positive regulator of the effects of progesterone, while PGR-A serve to antagonise the effects of PGR-B.[14] When progesterone binds to the ligand-binding domain of PGR, the receptor initiates a series of conformational changes, and it is released from the chaperone proteins to finally enter into the nucleus. In the nucleus, PGR dimerises to form homodimers (AA, BB) or heterodimers

Figure 1: Three isoforms of classical progesterone receptors.



PGR-B (120 kDa), but not PGR-A (94 kDa), includes 164 additional amino acids in the NTD (shown as BUS), where the AF3 domain and multiple phosphorylation sites are located. The NTD also contains an activation factor domain (AF1), which is common for PGR-B and PGR-A. The protein tertiary structure results in a folding at the H region between the DBD and LBD. The green bars in the DBD represent zinc-finger motifs. Post-translational phosphorylation (shown as green ellipses), acetylation (shown as violet triangle), and SUMOylation (shown as green hexagon) can occur basally or in response to ligand binding and affect PGR transcriptional activity. The numbering reflects amino acid residue positions.

AF1: first activation factor; AF2: second activation factor; AF3; third activation factor; BUS: B-upstream segment; DBD: DNA-binding domain; LBD: ligand-binding domain; NTD: N-terminal domain; PGR: progesterone receptor.

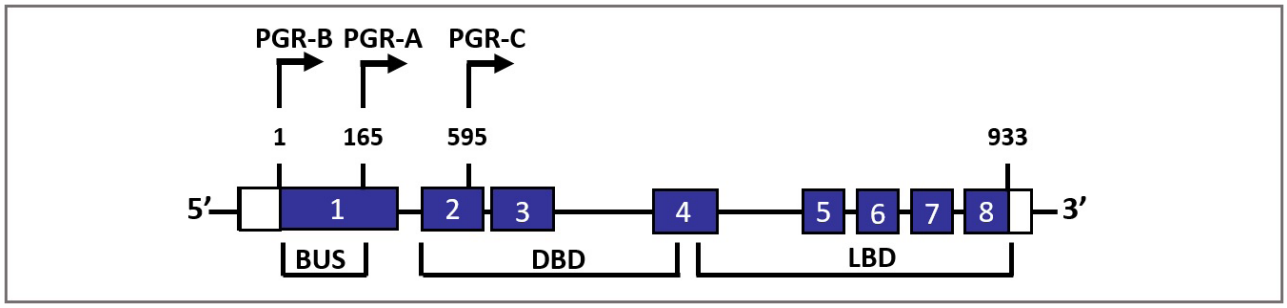
(AB), and binds to the progesterone response element sequence in the target gene.[14-19] A third variant of PGR, PGR-C isoform (60 kDa), has also been described in humans (Figures 1 and 2). PGR-C also can form heterodimers with PGR-A and PGR-B and regulates their transcriptional activity.[20] Such diverse possibilities of dimerisation of PGR potentially gives rise to a wide variety of physiological responses. The binding of PGR dimer to progesterone response elements follows their recruitment to coregulators (coactivators or corepressors) and regulation of the subsequent PGR-mediated target gene expression in an isoform-specific manner.[14,19]

Figure 3 shows a schema of canonical mechanism of PGR action. PGR isoforms interact with one another and mutually regulate their own activity. For example, PGR-A inhibits PGR-B action by its inhibitory domain and, thereby, PGR-A decreases the effects of progesterone on its target cells.[14,19] Additionally, tissue-specific coregulator expression along with subnuclear localisation of PGR and coregulator association may mediate tissue-specific progesterone action.[14,19,23]

Non-classical Progesterone Receptors

Non-classical PGRs are usually located on the cell surface as single transmembrane receptors. These receptors belong to G protein-coupled receptor superfamily and are associated with tyrosine kinase activity. Non-classical PGRs are of two types: membrane progestin receptors (mPR) family, also named the progestin and adipoQ receptor (PAQR), and the progesterone

Figure 2: Schematic representation of genomic configuration of three classical progesterone receptor isoforms and splice variants.



The nuclear PGR gene is composed of eight exons with 3100-bp coding regions and 5'- and 3'-untranslated regions. PGR-B and PGR-A isoforms are transcribed from two alternate transcription initiation sites and are identical to amino acids 165–993. PGR-C (60 kDa) isoform results from an in-frame initiation of translation and lacks exon 1.

BUS: B-upstream segment; DBD: DNA-binding domain; LBD: ligand-binding domain; PGR: progesterone receptor.

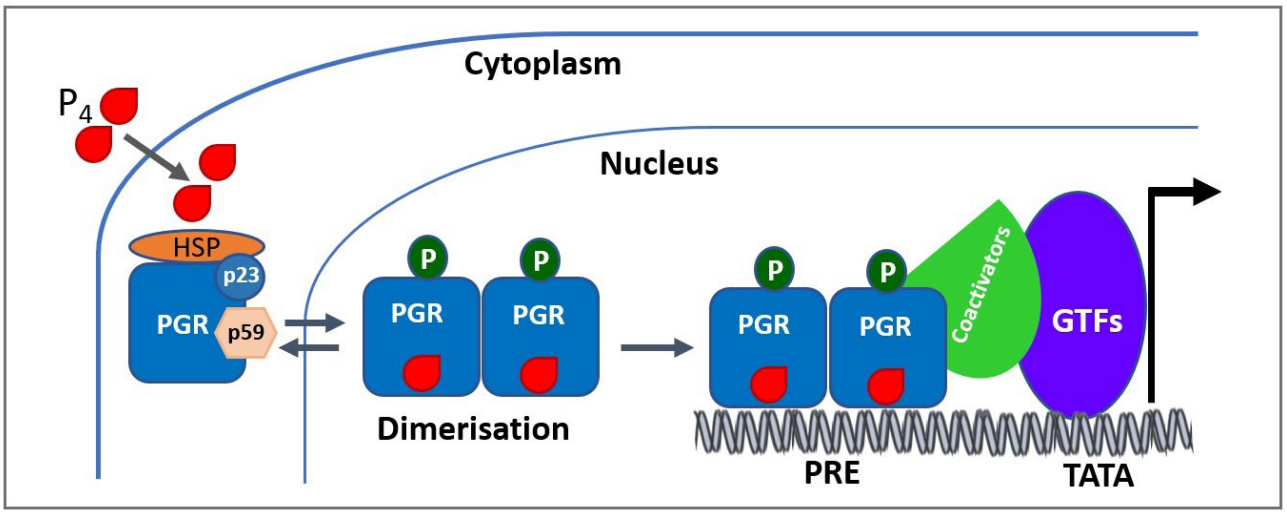
receptor membrane component (PGRMC) family, both having several subtypes.[8,11,16,19,24] There is evidence to suggest that both types of non-classical PGRs may interact to mediate their actions in target cells. The physiological significance of non-classical PGRs in the uterus is not clear. However, as discussed in the following section, these receptors are associated with the menstrual phase specific functions of uterine cells.[8]

Progesterone Receptors in Endometrium

Both classical and non-classical PGRs exhibit differential expression depending on endometrial cell type, and the phase of the menstrual cycle (Figure 4). Both PGR-A and PGR-B are expressed in the endometrial epithelium during early to mid-luteal phase, seemingly in preparation of embryo implantation.[18,19] The inhibitory effect of PGR-A on the expression of PGR-B causes negative regulation of the action of PGR-B, and thereby promotes hyperplasia and inflammation in this tissue.[18,19] During implantation, however, the expression level of PGR-A drops, while that of PGR-B remains constant to support the secretory function of glands of endometrium *functionalis*. On the other hand, PGR-A is the dominant isoform in endometrial stromal cells throughout the luteal phase and provides support to decidualisation, which is integral to the implantation process.[16-19] In fact, very low expression of both isoforms in endometrial cells may result in unexplained infertility and implantation failure. PGR knockout experiments in mouse studies demonstrated that the expression of PGR-A, but not of PGR-B, is obligatory for successful implantation and establishment of pregnancy.[25] On the other hand, the overexpression of PGR-A results in uterine enlargement and endometrial hyperplasia.[8,10,18] Thus, the ratio of PGR-A-to-PGR-B appears critical for the normal response of endometrium to progesterone.[26]

Among non-classical PGRs in humans, transcript levels of PGRMCs, mPRα, mPRγ, and mPRε fluctuate depending on the menstrual cycle phase. For example, levels of messenger RNAs (mRNA) for PGRMC1, mPRγ, and mPRε are upregulated during the proliferative phase and progressively decrease during the secretory phase, whereas mRNAs for mPRα and PGRMC2 are higher in the secretory phase along the increasing levels of luteal progesterone.[8,16,19,24] On the other hand, the levels of mPRβ, which are relatively higher in the human endometrium than that of mPRα, do not change significantly during the menstrual cycle; however, its expression is critical on Days 10–14 of the human menstrual cycle, as revealed in patients with a history of recurrent spontaneous abortion.[16,19]

Figure 3: A simplified scheme of the mechanism of progesterone receptor activation.



Ligand-free PGRs are present as inactive complexes associated with HSPs and chaperone proteins (p23 and p59). When progestin binds to the PGR, it undergoes conformational changes along with dissociation of HSPs and chaperone proteins (p23 and p59). PGRs then undergo dimerisation and bind to the HRE in the target DNA. Ligand-dependent conformational changes allow for the recruitment of cofactors and other GTFs to the promoter, producing a transcriptionally active complex that can direct gene transcription.

Adapted from Mani S, Portillo W.[21] *and Hill KK et al.*[22]

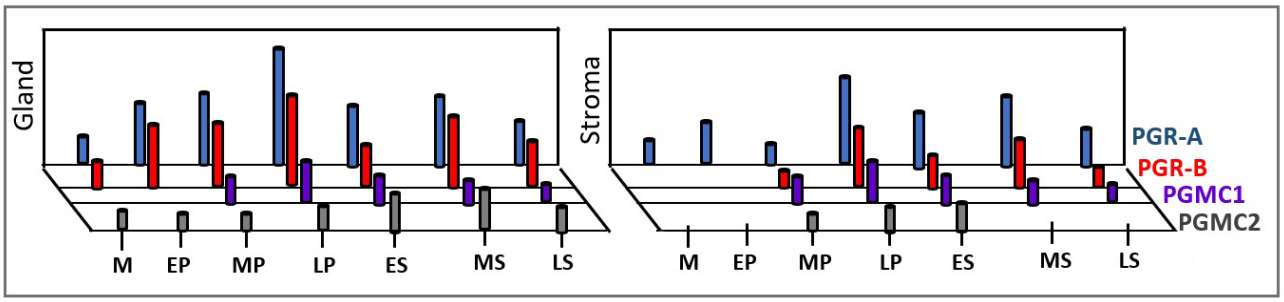
GTF: general transcription factors; HRE: hormone response element; HSP: heat shock protein: P_4: progesterone; PGR: progesterone receptor; PRE: progesterone response element.

As seen in Figure 4, PGRMC1 and PGRMC2 show inverse expression patterns during the menstrual cycle. PGRMC1 displays regulatory action on PGRMC2. PGRMC1 is involved in cell proliferation in the endometrium during the proliferative phase of the menstrual cycle.[16,19] On the other hand, PGRMC2 inhibits cell proliferation and supports endometrial differentiation during the secretory phase of the menstrual cycle.[16,19,24] Thus, the reported inverse profiles of PGRMC1 and PGRMC2 in the glandular and stromal compartments of human endometrium during the menstrual cycle appears important for endometrial growth and maturation. Several lines of evidence indeed indicate that both PGRMC1 and PGRMC2, and their expression ratio in endometrial epithelial and stromal components, are critical for endometrial preparation for successful pregnancy.[16,24]

PHYSIOLOGICAL BASIS OF PROGESTERONE RESISTANCE

In a case report, Keller et al.[27] reported on a 23-year-old female with initial complaint of infertility, who demonstrated inadequate endometrial maturation during luteal phase consistent with inadequate corpus luteum syndrome; however, she had a normal serum pattern of progesterone, oestradiol, follicle-stimulating hormone, luteinising hormone, and their cytosol-binding proteins. Exogenous progesterone did not correct the abnormality. Further investigation revealed inadequate maturation of her endometrium that caused from a markedly reduced number PGR in the target cells.[27] Thus, it appeared that the condition of 'pseudocorpus luteum insufficiency' could have occurred due to progesterone resistance at the receptor level in the target cells of endometrium.[28] Similar defect had been reported in a subgroup of females with infertility.[29]

Figure 4: Expression pattern of classical progesterone receptor-A and progesterone receptor-B and membrane receptors progesterone receptor membrane component 1 and progesterone receptor membrane component 2 in glandular (shown as "Gland") and stromal compartment (shown as "Stroma") of human endometrium in a typical menstrual cycle.



Marked expression of PGR-A and PGRR-B in the LP and at MS is notable. PGRMC1 and PGRMC2 show subtle differential expression patterns during the menstrual cycle.

Adapted from Reis FM et al.[8]

EP: early proliferative phase; ES: early secretory phase; LP: late proliferative phase; LS: late secretory phase; M: menstrual phase; MP: mid-proliferative phase; MS: mid-secretory phase; PGR: progesterone receptor; PGRMC: progesterone receptor membrane component.

Gene polymorphisms and epigenetic modifications to PGR may, theoretically, cause such progesterone resistance. Furthermore, anomalies in down-stream signalling elements, target genes, and regulator modules that are directly and indirectly linked to progesterone actions may cause functional progesterone resistance. Both types of progesterone resistance may be constitutively present in the target tissue or may be acquired by the target cells.[9,10,30,31] In the following sections, the authors discuss how both types of progesterone resistance are associated with endometriosis.

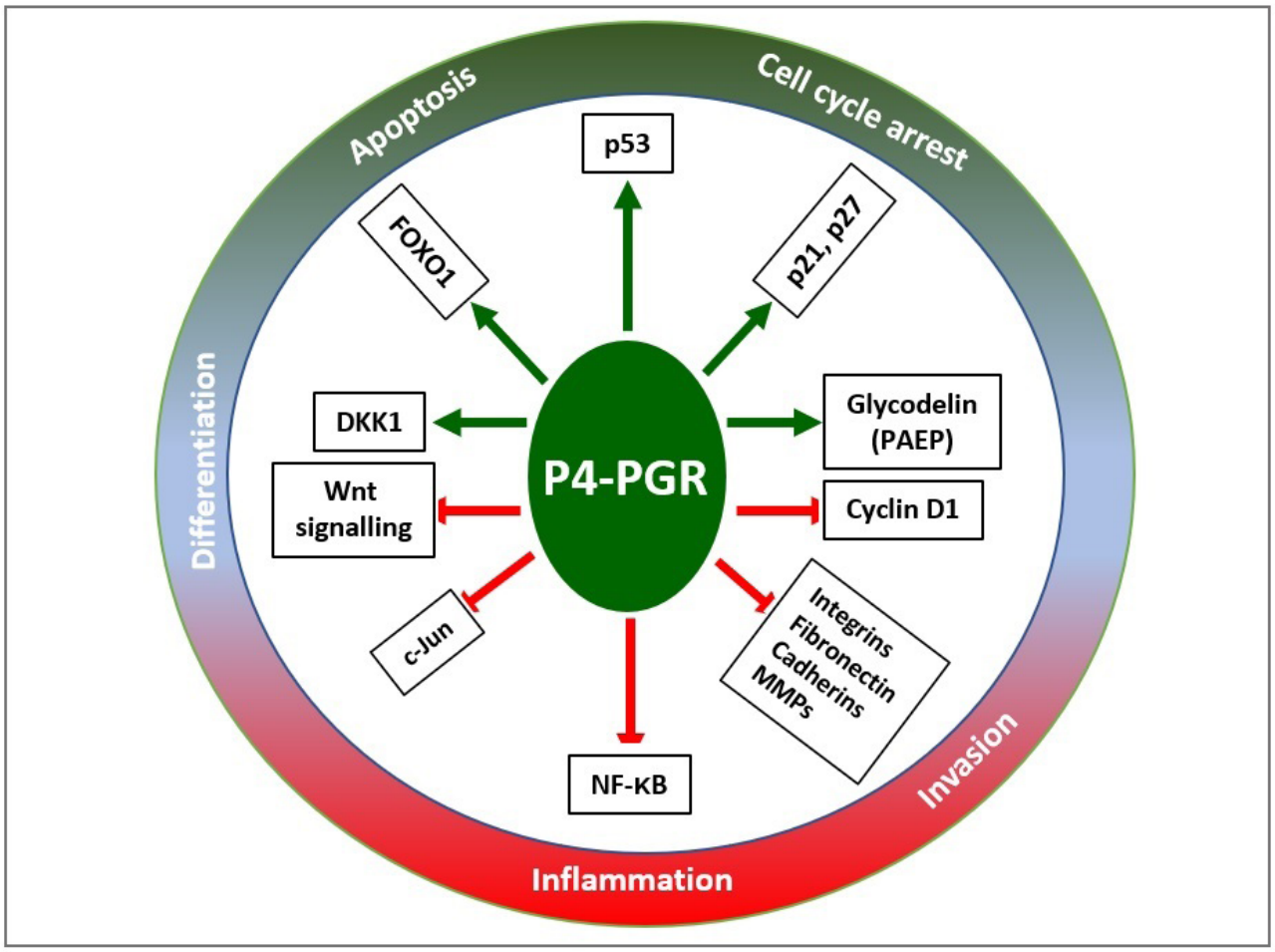
PROGESTERONE RESISTANCE IN ENDOMETRIOSIS

Ectopic Tissue

There is substantial evidence to suggest that progesterone resistance is a general feature of endometriotic lesion. Table 1 lists some of the supporting studies. As mentioned above, progesterone treatment could not induce the conversion of oestradiol to oestrone in ectopic tissue.[32] The enzyme 17β-HSD2, which is responsible for catalysing the reaction, is upregulated by progesterone in secretory epithelial cells of normal endometrium, but not by ectopic cells, providing the first-line evidence of progesterone resistance in endometriosis.[5] In fact, PGR concentrations, for both PGR-A and PGR-B, in endometriosis tissue are generally repressed.[6,33,34,44] Thus, it appears that attenuated PGR may result in altered gene transcription in ectopic tissue, even when progesterone concentration in circulation is normal.

Several large-scale gene expression studies revealed significant differences between ectopic and matching eutopic tissue. Many of the genes with differential expression were indeed progesterone target genes.[37,38,42,43,45] In this regard, it is notable that there are reports of increased PGR expression in endometrioma, with a higher or unchanged PGR-B expression in endometrioma compared with normal endometrium.[36,41] Interestingly, PGR expression was reportedly lower than in endometrium in primary endometriotic lesions, while in recurrent lesions there was no difference in PGR expression as compared with eutopic endometrium.[46] In another study, lower PGR expression was observed in stromal cells of ectopic lesions compared with eutopic tissue, while epithelial cells of ectopic lesion showed higher PGR expression in late secretory phase.[35]

Figure 5: Progesterone mediated networks of regulatory pathways that promote (shown as the green arrow) cell cycle arrest, apoptosis, and differentiation, and inhibit (shown as the red blocked arrow) inflammation and invasion.



Adapted from Yang S et al.[67]

DKK1: Dickkopf-related protein 1; FOXO1: Forkhead box protein O1; MMP: matrix metalloproteinase; NF-κB: nuclear factor κ-light-chain-enhancer of activated B cells; PAEP: human placental protein-14; PGR: progesterone receptor.

Eutopic Tissue

It is evident from the above discussion that ectopic tissue of females with endometriosis display suppressed PGR expression. The question whether a similar PGR suppression exists in the eutopic endometrium of females with endometriosis is rather unsettled. Table 2 provides a list of studies and the summary of results therein reflecting inconsistencies in this regard.

In a large-scale gene expression study, a higher clustering between proliferative and secretory phase samples from eutopic endometrial biopsies as compared with normal endometrium was observed, and it was associated with a substantial number of PGR target genes being affected.[48] Collectively, these results were suggestive of inadequate progesterone-mediated transition of late proliferative to the early secretory phase in females with endometriosis.

Furthermore, there are studies indicating a decrease in the PGR-B:PGR-A ratio along with relatively high PGR-A expression in eutopic endometrium compared with normal

Table 1: A chronicle of reports regarding progesterone resistance in endometriotic lesion.

Name with year	Major relevant observation
Vierikko et al.[32]	Lack of induction of 17β-HSD activity by progesterone, medroxy-progesterone acetate, or danazol was observed in endometriosis tissue along with lower concentration of PGR.
Prentice et al.[33]	Quantitatively lower immunopositive PGR expression was observed in endometriotic tissue compared to paired eutopic endometrium.
Bergqvist et al.[34]	Significantly lower expression of immunopositive PGR was observed in epithelial cells of ovarian endometriosis than in endometrial epithelial cells, but not in stromal cells.
Jones et al.[35]	Lower PGR expression in stromal cells of ectopic lesions compared with eutopic tissue, while epithelial cells of ectopic lesion showed higher PGR expression only in the late secretory phase.
Zeitoun et al.[5]	Deficient 17β-HSD2 expression, which is regulated by progesterone, was observed in endometriosis.
Misao et al.[36]	Dominant expression of PGR-B mRNA in ovarian endometriosis.
Attia et al.[6]	PGR-A but not PGR-B was expressed in endometriosis.
Matsuzaki et al.[37,38]	Significantly higher levels of PGR regulated 17β-HSD2 mRNA in epithelia of ectopic lesions of ovarian endometriosis compared with matched eutopic endometrium in secretory phase of menstrual cycle of ovarian endometriosis. No such difference was observed in deep endometriosis. In fact, 17β-HSD2 expression was not detected in either epithelial or stromal cells of 50% ectopic samples.
Wu et al.[39]	Large-scale transcriptional characterisation of differences between eutopic and ectopic endometrium revealed 904 differentially expressed genes contributing to 79 pathways, with over 100 genes with known functions, including PGR dependent signalling systems (Wnt and MAPK signalling).
Bukulmez et al.[40]	Increased expression of PGR-C mRNA relative to PGR-A and PGR-B mRNA was observed in ovarian endometriosis compared with eutopic and control endometrium. The PGR-A protein was barely detectable in endometriomas. The significance of the observations lies in the fact that PGR-A and PGR-B serve an anti-inflammatory role in the uterus by antagonising NF-κB activation and COX-2 expression, while PGR-C expression, which antagonises PGR-B, is associated with inflammation.
Smuc et al.[41]	Expression analysis revealed no significant difference in expression of PGR-A and PGR-B in ovarian endometriosis compared with control endometrium despite indication of selective progesterone resistance (AKR1C1 and AKR1C3).
Khan et al.[42,43]	Genomic expressional profile of ectopic tissue differs from that of eutopic, suggestive of differential regulation in genes involved in physiological functions including progesterone action in inflammation, cell cycle, and death, along with relative downregulation of PGR in the secretory phase of ectopic endometrium, which is suggestive of relative suppression of progesterone action in ectopic lesion.
Bedaiwy et al.[44]	Abundance and localisation of progesterone receptor isoforms in endometrium in females with and without endometriosis and in peritoneal and ovarian endometriotic implants revealed PGR-A as the predominant isoform in peritoneal endometriosis, while both PGR-A and PGR-B were detected in ovarian endometriosis. However, PGR-A levels were significantly elevated in ovarian endo-metriosis compared with peritoneal endometriosis.

17β-HSD: 17-β-hydroxysteroid dehydrogenase; 17β-HSD2: 17-β-hydroxysteroid dehydrogenase Type 2; AKR1C1: aldo-keto reductase family 1 member C1; AKR1C3: aldo-keto reductase family 1 member C3; COX-2: cyclo-oxygenase-2; MAPK: mitogen-activated protein kinase; mRNA: messenger RNA; NF-κB: nuclear factor κ-light-chain-enhancer of activated B cells; PGR: progesterone receptor.

Table 2: Summary of selected reports regarding progesterone resistance in eutopic endometrium during endometriosis.

Name with year	Major relevant observation
Jones et al.[35]	No marked difference in PGR expressions between normal endometrium and eutopic endometrium during endometriosis.
Attia et al.[6]	PGR-A and PGR-B detected in eutopic endometrial samples, with increased levels in the pre-ovulatory phase with near normal cyclical variation.
Igarashi et al.[47]	Eutopic endometriotic endometrium of proliferative phase showed significantly lower PGR-B:PGR-A ratio than that in normal endometrium.
Matsuzaki et al.[38]	In eutopic endometrium from patients with deep endometriosis, 17β-HSD2 expression in epithelial cells was significantly increased during the early, middle, and late secretory phases compared with the late proliferative phase. No such difference was detected in control endometrium.
Burney et al.[48]	Transcriptome analysis revealed reduced progesterone response in the transition from the proliferative to secretory phases in eutopic endometrium of females with endometriosis compared with normal endometrium.
Aghajanova et al.[49,50]	Isolated hESF from mid-secretory endometrium with and without endometriosis passaged *in vitro* and exposed to 8-Br-cAMP or progesterone displayed lower expression of decidualisation markers (IGFBP1 and prolactin) by hESF cells from females with endometriosis versus those without endometriosis in response to 8-Br-cAMP but not to progesterone. Decreased 3β-HSD1 and 17β-HSD2, and increased 17β-HSD1 with a shift towards an estrogenic milieu in hESF cells of eutopic endometrium of endometriosis. The normal response of hESF to progesterone, which involves a tightly regulated kinetic cascade of PGR and MAPK signalling pathways, resulting in deciualisation was not established by progesterone in hESF cells of endometriosis.
Gentilini et al.[51]	Both PGR-A and PGR-B expressed in endometrial stromal cells derived from females with and without endometriosis and grown as monolayers on plastic in 10% FBS containing medium was comparable.
Zelenko et al.[23]	The study revealed a blunted proliferative-to-secretory transition in early secretory phase endometrium of endometriosis, sugges-tive of progesterone resistance in endometrium of females with endometriosis.
Bedaiwy et al.[44]	In eutopic endometrium, levels of PGR-A were significantly elevated in females with endometriosis compared with females without disease, regardless of menstrual phase. Endometriotic lesions and eutopic endometrium from females with endometriosis are uniform in a PGR-A-dominant state.
Barragan et al.[30]	eSFs from endometriosis displayed a pro-inflammatory and progesterone resistance phenotype not detected in normal eSFs. The progesterone resistance in eSFs inherited from endometrial mesenchymal cells in endometrosis.
Anupa et al.[52]	Higher expression of 17β-HSD1 and PGR-A in eutopic endometrium in endometriosis compared with normal endometrium, particularly during the secretory phase of the menstrual cycle. Dysregulated 17β-HSD1 expression along with alterations in the PGR-A:PGR-B ratio, resulting in hyperoestrogenism and progesterone resistance during the secretory phase of the menstrual cycle, rather than an anomaly in aromatase expression as hallmarks of eutopic endometrium of patients with ovarian endometriosis who are infertile. Also, revealed that fertility and menstrual cycle histories exert differential effects on steroid physiology in endometrium from endometriosis patients compared with control subjects.

8-Br-cAMP; 8-bromoadenosine 3',5'-cyclic monophosphate; 17β-HSD1: 17-β-hydroxysteroid dehydrogenase Type 1; 17β-HSD2: 17-β-hydroxysteroid dehydrogenase Type 2; eSF: endometrial stromal fibroblasts; FB: fibroblast; hESF: human endometrial stromal fibroblasts; IGFBP1: insulin-like growth factor-binding protein 1; MAPK: mitogen-activated protein kinase; PGR: progesterone receptor.

endometrium.[44,47,53] On the contrary, several studies failed to substantiate these findings. A cyclical variation in PGR expression in eutopic endometrium from females with endometriosis, which was comparable to normal cyclical endometriumm was reported in an early report.[34] Further, several studies failed to mark any notable difference in the expression of PGR-A and PGR-B expression in normal and eutopic endometrium.[6,35,51]

The reported inconsistencies in results of the previous studies on endometrial progesterone receptivity might have resulted from differences in the technical details (e.g., details of tissue collection and handling) and the lack of a categorical consideration of the relative effects of fertility and menstrual histories on PGR expression and actions in the endometrium of patients with and without endometriosis.[52] Moreover, the reported studies on PGR response used isolated cells maintained in a 2D culture system, which might be an inadequate model for addressing the core issue of progesterone resistance due to the fact that such isolated cells often lose their differential behaviour typically seen in the tissue.[31,49,50,54]

Additionally, the statistical design and clinical details in a few studies were not foolproof. The Endometriosis Phenome (and Biobanking) Harmonisation Project (EPHect) guidelines highlight the necessity of developing a consensus on the standardisation and harmonisation of phenotypic surgical and clinical data and biological sample handling methods in endometriosis research.[55,56] In a recent controlled study conducted according to EPHect guidelines, lower levels of expression of aromatase and oestrogen receptor β along with higher 17β-HSD1 and PGR-A in endometrium of females with ovarian endometriosis was observed.[52] Thus, dysregulated expression of 17β-HSD1 and PGR results in hyperoestrogenism and progesterone resistance during the secretory phase of the menstrual cycle, rather than an anomaly in aromatase expression, was the hallmark of eutopic endometrium from patients with ovarian endometriosis.[52] It is now evident that fertility and menstrual cycle histories exert differentiating effects on endometrial physiology in females with endometriosis, vis-à-vis normal healthy endometrium.[42,43,52]

MECHANISMS OF PROGESTERONE RESISTANCE IN ENDOMETRIOSIS

Collectively, it appears from different lines of evidence available that progesterone resistance in endometriosis is not an 'all-or-none' phenomenon, and that ectopic tissue exhibits higher order of progesterone resistance than eutopic tissue in a relative scale. Suppression of PGR expression and activity in target cells may potentially take place due to interference in transcriptional, post-transcriptional, and post-translational events, and at the level of protein stability. These events can reportedly be affected in endometriosis;[23,57-61] however, progesterone receptor polymorphisms are not related to susceptibility to endometriosis.[62] Fundamentally, it depends on genetic background, natural history of development of the individual, and the organ and macro- to micro-environmental details.

In an interesting study, Jackson et al.[63] demonstrated that PGR-A and PGR-B expression in the eutopic endometrium markedly reduced over time, between 3 and 15 months, after induction by using a model of experimental induction of endometriosis and implanting endometrial tissue into the peritoneal environment of a baboon.[63] In connection to this, McKinnon et al.[10] presented an elegant model explaining how constant exposure to the inflammatory ecology in peritoneal environment may result in suppression of PGR expression and activity. It now appears that human endometrial fibroblasts display PGR resistance and an inflammatory phenotype, possibly due to epigenomic modifications in the endometrium during endometriosis.[10,23,30,31,40,61] Although PGR gene expressions and PGR actions are significantly dysregulated in both eutopic and ectopic tissues compared with control endometrium, the underlying epigenetic mechanisms for PGR suppression in the eutopic tissue are different from ectopic tissue.[57,64]

IMPLICATIONS OF PROGESTERONE RESISTANCE IN ENDOMETRIOSIS

Progesterone resistance in endometriosis has been implicated in four clinically challenging trade-offs: pain, infertility, inflammatory disorders, and neoplasm; these impair the quality of life of the patients.[65] Nearly 10% of females

of reproductive age have endometriosis, with more than 70% of them affected by chronic pelvic pain.[65] Endometriosis is also a well-acknowledged cause of infertility, which is seen in 50% of patients with endometriosis with normal ovulation and normo-spermic partners. Severe endometriosis is associated with poor embryo implantation rates and pregnancy rates in women undergoing *in vitro* fertilisation treatment.[65] Females with endometriosis often have several inflammation-linked and other comorbidities like uterine fibroid, adenomyosis, pelvic inflammatory disorder, inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, fibromyalgia and cardiovascular diseases.[65] Endometriosis is generally considered benign; however, unmanaged endometriosis with atypia may result in ovarian and extra-ovarian cancers.[66]

Based on generally known progesterone actions in target tissues as discussed above and shown in Figure 5, it appears that resistance of progesterone action may cause the above-mentioned endometriosis-associated conditions in patients.[21,67] Yet, progestin treatment is met with failure in a large number of patients.[7,8] Is it possible to overcome this resistance with the help of combinatorial therapies? For example, can the addition of a DNA methyltransferase inhibitor to reverse epigenetically suppressed PGR expression be helpful? The epigenetic modifications of PGR and progesterone target genes in eutopic and ectopic tissues may, however, be markedly different.[57,64]

In primary endometriotic lesions, PGR expression is lower than in endometrium, generally having better prognosis with progesterone treatment. Meanwhile, there was no difference in PGR expression as compared with eutopic endometrium in recurrent lesions, generally having poor prognosis with progesterone treatment.[46]

It is evident from the above-discussion that PGR (both PGR-A and PGR-B) expression status is not sufficient to understand the nature of progesterone resistance. Can there be a set of novel and more useful functional parameters? How is PGR expression and response in target cells from females with endometriosis affected by other linked molecular mechanisms?[11,21,26,68,69] Several such unmet questions point to the fact that there is an urgent need for a better understanding of the nuanced characteristics of progesterone resistance in ectopic and eutopic tissue in endometriosis, to innovate better management and treatment of progesterone resistance in the future.

CONCLUSION

It has been more than half a century since the administration of progestins has become a part of the normalised procedure to treat endometriosis. Nevertheless, its success rate is limited as it fails with time and some patients do not respond to this therapy as expected. As the authors have discussed, such progesterone resistance occurs due to supressed PGR expression, dysregulated downstream PGR actions, or both. In any case, estimated upscaling of dosage schedule or by small modifications in the molecular design of the drug cannot help in circumventing these issues. Success with available combinatorial approach is also not very promising. There is an urgent necessity for deeper and better understanding of the cellular and molecular issues related to progesterone resistance in endometriosis so that novel approaches may be innovated to restore the various homeostatic mechanisms disrupted by progesterone resistance in ectopic and eutopic tissues in endometriosis.

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The Association of Serum Progesterone Levels on Day of Oocyte Retrieval with Pregnancy Outcome

Author:	*Parul Garg Sarvodaya Fertility and IVF Centre, Delhi, India *Correspondence to drparul23@yahoo.co.in

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Abstract

Aims and Objectives: To establish the level of serum progesterone (P4) on the day of oocyte retrieval beyond which it can affect the outcome of *in vitro* fertilisation (IVF), and to further establish the incidence of serum P4 rise in an agonist and antagonist cycle.

Methods: This prospective observational cohort study was conducted from November 2020 to November 2021 at the Sarvodaya Fertility and IVF Centre, Delhi, India. For this study, the author recruited 352 couples with infertility who were treated with IVF/intracytoplasmic sperm injection-embryo transfer, of which 279 patients completed an IVF/intracytoplasmic sperm injection-embryo transfer cycle during the study period and were included in the final analysis.

The standard gonadotropin-releasing hormone antagonist (fixed or variable) and long gonadotropin-releasing hormone agonist controlled ovarian stimulation protocols were used in all patients. Participants were recruited if they were undergoing controlled ovarian stimulation with all gonadotropins, recombinant follicle-stimulating hormone/urinary human menopausal gonadotropin, or recombinant luteinising hormone. The study population was sub-grouped into two groups according to their P4 level on day of oocyte retrieval (calculated according to receiver operating characteristics curve): Group A (p≤11.6 ng/dL; n=247 out of 27; 88.5%) and Group B (p>11.6 ng/dL; n=32 out of 279; 11.5%). Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) 17.0 version (IBM, New York City, New York, USA).

Results: The percentage of patients with a rise in P4 on the day of oocyte retrieval were found significantly more in the antagonist protocol (13.3% [24 out of 181]) than in the agonist protocol (8.2% [8 out of 98]; p=0.04). Pregnancy rate was significantly higher in Group A (39.3% [97 out of 247]) compared with Group B (12.5% [4 out of 32]). The clinical pregnancy rate was also significantly higher in Group A (34.4% [85 out of 247]) compared with Group B (6.3% [2 out of 32]).

Conclusion: Patients with higher levels of P4 (>11.6 ng/mL) were associated with lower pregnancy and clinical pregnancy rates.

Key Points

1. In this prospective observation cohort study, the author recruited 352 couples with infertility, who were treated with *in vitro* fertilisation/intracytoplasmic sperm injection-embryo transfer, to determine if the level of serum progesterone (P4) on the day of oocyte retrieval could affect the outcome of *in vitro* fertilisation treatment.

2. An increase in P4, along with high oestradiol levels, could lead to endometrial glandular stromal asynchrony and implantation failure.

3. Identifying the threshold where P4 affects pregnancy outcomes is important as it could aid a practitioner in counselling their patient.

INTRODUCTION

Despite the use of gonadotropin-releasing hormone (GnRH) analogues, subtle increases in serum progesterone (P4) levels beyond an arbitrarily defined threshold value have been observed at the end of the follicular phase in controlled ovarian stimulation (COS) cycles for *in vitro* fertilisation (IVF). Although the frequency of elevated serum P4 level varies in COS cycles, incidences as high as 35% (5–35%) have been reported in individuals treated with GnRH agonists and 38% (9–38%) in those treated with GnRH antagonists.[1-6]

In natural cycles, there is a small physiological rise in serum P4 levels prior to ovulation and it is thought to be essential for bringing about an increased luteinising hormone (LH) receptor induction on granulosa cells for enhanced LH action. This rise in serum P4 levels is due to the increased responsiveness of granulosa cells to LH. However, the rise seen in stimulated cycles is far more than that in natural cycles, possibly due to higher number of follicles (stimulated by maintained high follicle-stimulating hormone [FSH] concentrations as a result of daily FSH injections). These follicles will produce and secrete more P4 into the ovarian vein than a single follicle in the normal mid-follicular phase, but with declining FSH concentrations.

In the event of ovarian stimulation-induced multiple-follicle growth, the P4 output to the periphery will be magnified in accordance with the number of follicles and the FSH drive. This is likely to have an impact on the concentration of P4 in the periphery and may influence endometrial development. Thus, the three major components to the degree of P4 secretion from the ovaries will be the number of follicles (or granulosa cells); the degree of trophic stimulus (FSH drive to granulosa cells); and the degree of LH drive to theca cells, which encourages conversion of P4 precursors to androgens and then oestrogen.[6]

The rise in serum P4 levels then coupled with high oestradiol levels may result in endometrial glandular stromal asynchrony, which may be associated with implantation failure due to a luteal phase defect, while having no influence on oocyte or embryo development.[7-10]

A large number of clinical studies have examined the effect of a rise in serum P4 levels on the day of human chorionic gonadotropin (hCG) administration in GnRH agonist and antagonist cycles on pregnancy rates. The results of these studies are variable. Possible explanations for the discrepancies in the findings are the use of retrospective study design, use of different protocols of controlled ovarian stimulation, and different cut-off levels for P4 at the time of data analysis. Some studies imprecisely define references for elevated serum P4 levels. Furthermore, there is variation in the statistical methods used to estimate specific circulating P4 limit values and in the precision of P4 measurements that use different immunoassays.[11] Most investigators have agreed on a cumulative deleterious effect on pregnancy rates as a result

of this supra-physiological rise in P4 in the late follicular phase.[12-20]

There are a few studies that have showed the association of the rise of P4 on day of oocyte retrieval (OCR) and IVF outcome. Nayak et al.[21] showed that implantation rate and pregnancy rate were significantly higher when serum P4 level is <12 ng/dL on the day of OCR in the antagonist cycle.

The day of OCR is a provocative time point, particularly in antagonist cycles as the agent used to prevent the LH surge is stopped 36 hours before OCR. However, the average half-life of the GnRH antagonist (GnRHant), when given in multiple doses, is approximately 20 hours, which ultimately leads to a rapid recovery from pituitary suppression. As a result of this, an endogenous LH surge may occur due to rapid recovery from pituitary suppression, along with an hCG trigger that may raise P4 levels proximally to the retrieval and the result may have a deleterious effect on the endometrial lining.[21] Despite this challenging possibility, there have been few studies that have sought to determine the normal distribution of P4 levels at 36 hours after an hCG trigger, or threshold P4 levels at this time point beyond which pregnancy is unlikely.[21-23]

AIMS AND OBJECTIVES

In this report, the author presents a prospective non-interventional, observational single centre cohort study, which aims to evaluate the association between serum P4 levels on day of OCR and pregnancy outcome in patients undergoing IVF with COS. This study also aimed to establish the incidence of serum P4 rise in agonist and antagonist cycles, as well as in cycles that use drugs such as recombinant FSH (rFSH) and rFSH versus human menopausal gonadotropin cycles.

MATERIAL AND METHODS

This prospectively observational cohort study was conducted from November 2020 to November 2021 at the Sarvodaya Fertility and IVF centre, Delhi, India. There were 352 couples with infertility who were treated by IVF intracytoplasmic sperm injection-embryo transfer (ICSI-ET) who met the inclusion criteria and were included in the study. Participants undergoing COS with all gonadotropins, rFSH/urinary hMG, or recombinant LH were recruited.

P4 levels were obtained at three time points: on Day 2 or 3 of IVF cycles; Day 6 of stimulation in long agonist as well as multiple dose antagonist protocols; and in the morning of OCR. The standard GnRHant (fixed or variable) and long GnRH agonist COS protocols were used for all patients.

Written informed consent was taken from all couples before recruiting the patients in the study.

Inclusion Criteria

All females registered for IVF or IVF/ICSI-ET using an agonist or antagonist protocol were included in this study.

Exclusion Criteria

Individuals were excluded if they had a P4 level >2 ng/mL; ≥2 previous failed IVF cycles; unclipped hydrosalpinx, intramural fibroid ≥4 cm, or localised (>4 cm) or diffuse adenomyosis; donor recipient cycles; were aged more than 37-years-old; or were on dehydroepiandrosterone acetate at the start of the stimulation.

Cancellation Criteria

Some patients were unable to continue in the study as a result of poor response (≤3 oocytes retrieved); endometrium being ≤6 mm on the day of OCR; having embryos with poor morphology on Days 2, 3, or 5; and the embryo transfer not being completed.

Outcome Measures

The primary outcome measure was the pregnancy rate in females undergoing IVF. However, the author also looked at several secondary outcome measures, including clinical pregnancy rate; pregnancy loss rate; ectopic pregnancy rate; incidence of serum P4 rise in various protocols; and incidence of serum P4 rise with cycles using drugs such as rFSH/LH versus only rFSH and rFSH versus hMG cycles.

The pregnancy rate included looking at the number of patients with serum β-hCG >20 IU/mL on Day 14 after OCR, divided by the total number of cycles. Meanwhile, the clinical pregnancy rate is the number of clinical pregnancies expressed per 100 completed cycles.

In this study pregnancy loss meant a miscarriage at 12 weeks. An ectopic pregnancy is where implantation takes place outside the uterine cavity.[24]

Hormonal Assay

Blood samples were drawn at three designated time points. The blood was collected aseptically and allowed to clot as soon as possible. No additives or preservatives were required to maintain integrity of the sample. The sample was analysed for P4 with the LIAISON® progesterone assay (DiaSorin, Saluggia, Italy), which is a chemiluminescent immunoassay to be used with the LIAISON analyser (DiaSorin) for quantitative determination of P4 in human serum.

STATISTICAL ANALYSIS

Sample Size Calculation

At the Sarvodaya Fertility and IVF Centre, the pregnancy rate in patients with infertility undergoing IVF ranges from 35–40%. For the sample size calculation, the author expected a pregnancy rate of 35%, with a precision error of estimation of 6% and an alpha of 0.05; therefore, a sample size of at least 245 cases was needed. However, the author had taken at least 300 cases to counteract any cases that dropped out. The sample size was calculated using this formula: $(Z^2 \times p \times q)/d^2$.

Statistical Methods

Statistical testing was conducted with the Statistical Package for Social Sciences (SPSS) 17.0 version (IBM, New York City, New York, USA). Continuous variables are presented as mean±standard deviation, while categorical variables are presented as absolute numbers and percentage. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired student's t test, whereas the Mann–Whitney U test was used for those variables that were not normally distributed. Categorical variables were analysed using either the chi-square test or Fisher's exact test. A receiver operating characteristics (ROC) analysis was calculated to determine the optimal cut-off values for (mention those variables name). The area under the curve and its 95% confidence interval. For all statistical tests, a p value of less than 0.05 was taken to indicate a significant difference.

RESULTS

In the author's study, 352 patients were recruited. During the study period, 279 patients completed an IVF/ICSI-ET cycle and were included in the final analysis. Seventy-three patients did not meet the final inclusion criteria because they either had poor response (n=11); their endometrial thickness on the day of OCR was ≤6 mm (n=4); the embryos were poor morphology (n=14); or they did not undergo immediate embryo transfer (ET) because of suspected ovarian hyperstimulation syndrome (n=42). Two patients did not undergo ET due to having a fever as a result of an Upper respiratory tract infection on the day of transfer. The protocols used in patients were either agonist (n=98) or antagonist (n=181). Forty-two patients out of 279 took LH in addition to rFSH for gonadotropin stimulation and 12 patients took only hMG for gonadotropin stimulation.

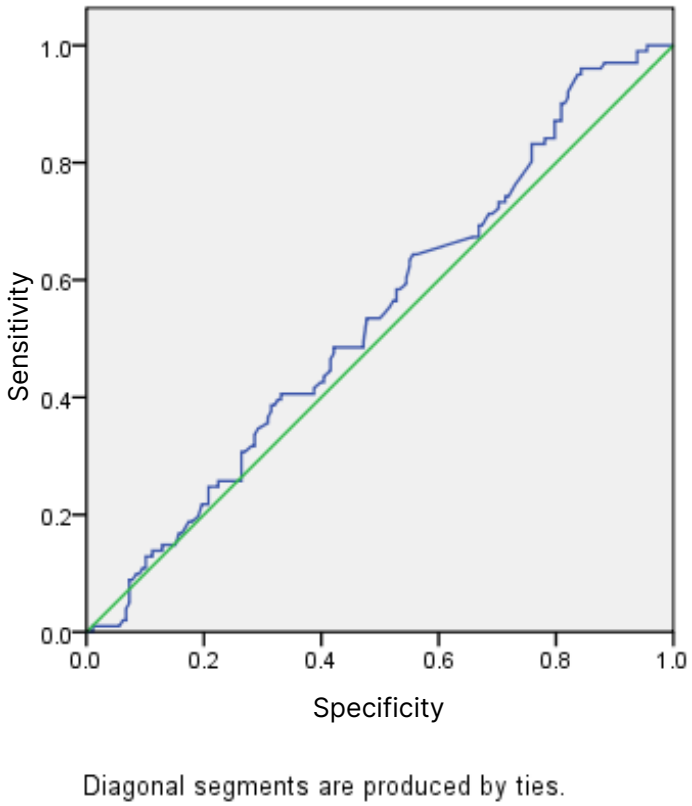
Only the patients with P4 levels of <2 ng/dL on Days 2 or 3 of the treatment cycles were recruited in the study. P4 levels on Day 6 of cycle were not associated with pregnancy outcome.

Using a ROC curve (Figure 1) on the day of OCR, the area under the curve was 0.537, with a 95% confidence interval. The serum P4 level above this it affects IVF outcome: 11.6 ng/dL.

The study population was sub-grouped into two groups according to P4 level on the day of OCR (calculated according to the ROC curve): Group A (p≤11.6 ng/dL; n=247 out of 279; 88.5%) and Group B (p>11.6 ng/dL; n=32 out of 279; 11.5%).

The demographic variables shown in Table 1 were similar in two groups.

Figure 1: Receiver operating characteristic curve on the day of oocyte retrieval.



The diagonal segments are produced by ties.

The percentage of subjects with P4 rise on the day of OCR were found significantly more in antagonist protocol (13.3% [24 out of 181]) than in agonist protocol (8.2% [8/98]; p=0.04). There was no association found in the rise of P4 with using rFSH alone versus the addition of recombinant LH (p=1.000) for superovulation and also in recombinant gonadotropins versus urinary hMG (p=0.635; Table 2).

Pregnancy rate was significantly higher in Group A (39.3% [97 out of 247]) when compared with Group B (12.5% [4 out of 32]). The clinical pregnancy rate was also significantly higher in Group A (34.4% [85 out of 247]) when compared with Group B (6.3% [2 out of 32]). Early pregnancy loss was not significantly different in both groups (p=0.728) and the incidence of ectopic pregnancy were similar in both groups (p=0.217; Table 3).

DISCUSSION

This prospective observational study was performed with 279 IVF/ICSI-ET cycles to establish the level of serum P4 on day of OCR to determine how it can affect the outcome of IVF and to further establish the incidence of serum P4 rise in an agonist and antagonist cycle, as well

Table 1: The demographic variables of patients Groups A and B.

Variables	Group A (P4≤11.6 ng/dL [n=247])	Group B (P4>11.6 ng/dL [n=32])	p
Age (years)	30.50±3.29	31.16±2.89	0.295
BMI (kg/m²)	26.26±4.84	24.55±3.47	0.053
Duration of infertility (years)	4 (1–16)	4 (1–12)	0.935
AMH (pmol/L)	27.50 (4.6–120.0)	21.55 (7.6–131.5)	0.251
Total dose of gonadotropins required (IU)	1,575 (700–3,525)	1,700 (875–4,000)	0.113
Duration of stimulation (days)	8.50±1.46	8.88±1.16	0.166
Terminal serum E2 (pg/mL)	1,400 (480–3,715)	1,801 (330–3,385)	0.110
Number of oocytes retrieved	10.72±4.24	11.97±4.43	0.119
Endometrial thickness on day of OCR (mm)	9.51±1.63	9.40±1.75	0.723
Number of embryos transferred	2.11±0.71	2.34±0.65	0.072

Data is presented as mean±SD or median as applicable.

AMH: anti-Müllerian hormone; E2: oestradiol; OCR: oocyte retrieval; SD: standard deviation; P4: progesterone.

as in cycles using drugs such as rFSH/LH versus only recombinant FSH and rFSH versus hMG cycles. Using the ROC curve, a serum P4 level of 11.6 ng/dL on day of OCR was identified as the most appropriate threshold to define detrimental levels of serum P4 for the outcome of IVF/ICSI-ET cycles.

It showed that P4 rise (>11.6 ng/dL; n=32) on the day of OCR was significantly more in the antagonist group (13.3% [24 out of 181]) compared with the agonist group (8.2% [8 out of 98]). This result is in consistent with the Nayak et al.[21] study, which evaluated the distribution of p levels on the day of OCR as it relates to the outcome of pregnancy in an antagonist protocol, which may be at higher risk for elevated p levels. The day of OCR is a provocative time point, particularly in antagonist cycles, as the GnRHant used to prevent the LH surge is stopped 36 hours before OCR. However, the average half-life of GnRHant (when given in multiple doses) is approximately 20 hours, which ultimately leads to a rapid recovery from pituitary suppression. As a result of this, an endogenous LH surge may occur due to rapid recovery from pituitary suppression, along with hCG trigger that may raise P4 levels to be more proximal to the retrieval and the result may have a deleterious effect on the endometrial lining.[21]

The primary reason why there were 91 patients treated with the agonist protocol and 181 patients were treated with the antagonist protocol was because, at the author's centre, the antagonist protocol is the most commonly used in patients. The patients using agonist protocol were included as none of the studies establish the incidence of serum P4 rise in an agonist and antagonist cycle. This is the only study that shows that P4 rise (>11.6 ng/dL; n=32) on the day of OCR was significantly higher in the antagonist group (13.3% [24 out of 181]) compared with the agonist group (8.2% [8 out of 98]).

Table 2: The differences between Groups A and B depending on the protocol and type of gonadotropin used.

	Group A (P4≤11.6 ng/dL [n=247])	Group B (P4>11.6 ng/dL [n=32])	p
Protocol			
Agonist (n=98)	90 (91.8%)	8 (8.2%)	0.04
Antagonist (n=181)	157 (86.7%)	24 (13.3%)	N/A
Types of gonadotropins			
rFSH (n=225)	199 (88.4%)	26 (11.6%)	1.000
rFSH+LH (n=42)	38 (90.47%)	4 (9.52%)	N/A
rFSH (n=267)	237 (88.8%)	30 (11.2%)	0.635
Urinary hMG (n=12)	10 (83.3%)	2 (16.7%)	N/A

hMG: human menopausal gonadotropin; LH: luteinising hormone; rFSH: recombinant follicle-stimulating hormone.

This study also showed that P4 rise (>11.6 ng/dL; n=32) on the day of OCR was associated with a significantly decreased pregnancy rate and decreased clinical pregnancy rate after fresh embryo transfer in females undergoing ovarian stimulation for IVF. The pregnancy rate observed was significantly higher in normal P4 level (≤1.6 ng/mL; n=247) group than in elevated P4 level (>11.6 ng/mL; n=32) group (39.3% [97 out of 247] versus 12.5% [4 out of 32]; p=0.003). The clinical pregnancy rate observed was significantly higher in normal P4 level (≤11.6 ng/mL; n=247) group than in elevated P4 level (>11.6 ng/mL; n=32) group (34.4% [85 out of 247] versus 6.3% [2 out of32]; p=0.001).

A study by Niu et al.[22] examined P4 levels on the day of OCR in cycles with GnRH agonists (long and short protocols) and found that P4 levels correlated with the number of oocytes and embryos but could not predict the outcome of the pregnancy. In a prospective study of 186 females, Nayak et al.[21] examined the prediction of assisted reproductive technology success depending on P4 level on the day of OCR in patients on a short stimulation protocol with GnRHant. Patients with a P4 rise (>12 ng/mL) on the day of OCR had lower pregnancy and implantation rates in this study.[20] Fernandez et al.[23] performed a prospective cohort study of 400 IVF/ICSI cycles, with a fresh embryo transfer on day 2or 3. They proposed a serum P4 level on percentile 90 as a threshold. Pregnancy rates were not affected; however, there were more miscarriages (25.7% versus 43.8%) and a lower live birth rate (28.0% versus 23.1%) in the P4 rise group, which is not statistically significant.[23] There was no statistically significant difference found in demographic variables and use of type of gonadotropins in this study.

The advantage of identifying a P4 threshold beyond which pregnancy outcomes may be affected can aid in the practitioner's ability to counsel a patient. Using time more proximally to OCR, the author discovered that a P4 level of >11.6 ng/mL may be predictive of significantly poorer pregnancy and clinical pregnancy rates.

These results have several important clinical considerations. Firstly, the negative effect of an elevated P4 at the time of OCR appears to be limited to the endometrium, as no effect on oocyte maturation or fertilisation rate was detected; this has been corroborated by previous studies in donor recipient IVF cycles as well as in frozen embryo cycles.[7-10] Therefore, a simple solution may be cryopreserving embryos when P4 levels exceed this threshold. Thus, the P4 level on the day of OCR may help to stratify which patients may benefit from embryo cryopreservation in lieu of a fresh ET. Secondly, this article hypothesises that there may be a benefit in continuing the GnRHant beyond the

Table 3: The outcomes of the study.

Outcomes	Group A (P4≤11.6 ng/dL [n=247])	Group B (P4>11.6 ng/dL [n=32])	p
Pregnancy rate	97 (39.3%)	4 (12.5%)	0.003
Clinical pregnancy rate	85 (34.4%)	2 (6.3%)	0.001
Early pregnancy loss	11 (4.4%)	1 (3.1%)	0.728
Ectopic pregnancy	1 (0.4%)	1 (3.1%)	0.217

P4: progesterone.

day of OCR, not only prevent the rise in P4 levels but to also prevent the potential cumulative negative effect that the premature rise may have on the endometrium after an hCG trigger. As the cumulative negative effect on the endometrium may be abated in this fashion and thus lead to an improvement in pregnancy rates; however, this has not yet been studied, and any potential adverse effects of the antagonist on the embryo would need to be considered.

CONCLUSION

This study found a significant correlation between the levels of P4 on the day of OCR and a positive outcome for assisted reproductive technology procedure. Patients with lower levels of P4 (≤11.6 ng/mL) were more likely to achieve pregnancy and deliver a healthy baby. In patients contemplating ET in favour of subsequent frozen ET, P4 levels at OCR may be one more piece of information that would impact their decision.

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Heterotopic Pregnancy after *IVF* and Embryo Transfer Post-unilateral Salpingectomy: A Case Report and Literature Review

Authors:	*Arjola Agolli, Hanyou Loh, Olsi Agolli Division of Clinical and Translational Research, Larkin Health System, Miami, Florida, USA *Correspondence to arjolamusta@hotmail.com

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Abstract

Heterotopic pregnancy (HP) is the simultaneous occurrence of intrauterine and ectopic pregnancies (EP). The incidence of HPs occurring spontaneously ranges from 1 in 10,000 to 1 in 30,000. However, this incidence is reported to be 1 in 100 pregnancies following artificial reproductive techniques. HP is a potentially life-threatening condition that is frequently misdiagnosed, as most diagnoses for HPs are delayed, and are only made after rupture of the EP. A high index of suspicion is, therefore, required for an accurate and timely diagnosis in order to reduce maternal morbidity and mortality, which currently stands at 1 in 200,000 live births. The most common risk factors include pelvic inflammatory disease, previous EP, assisted reproduction techniques, and ovarian hyperstimulation syndrome.

Transvaginal ultrasound is the gold standard for diagnosis. As detection of an intrauterine pregnancy often leads to the mistaken exclusion of a concomitant EP, a careful transvaginal scanning of the uterus and appendages should be performed in all females of reproductive age with a positive pregnancy test and red flags in anamnesis, and/or with clinical symptoms. Routine transvaginal ultrasound at Day 27 after embryo transfer could facilitate the diagnosis of HP; however, symptoms onset before or after Day 27 are clues to early diagnosis. MRI can be very helpful in diagnosing atypical cases.

Key Points

1. While heterotopic pregnancies occurring spontaneously is 1 in 10,000 to 1 in 30,000, the rate of incidence is 1 in 100 in females following artificial reproductive techniques.

2. Currently, transvaginal ultrasound is the gold standard for diagnosis, and scanning the uterus should be performed in all females who have a positive pregnancy test.

3. Heterotopic pregnancies can occur in any female of reproductive age, especially those who have undergone in vitro fertilisation, but are difficult to diagnose because of their rarity.

INTRODUCTION

Infertility is one of the major health concerns faced by individuals globally.[1] The 2021 United Nations (UN) World Population Prospects data sheet estimates that the average worldwide total fertility rate is 2.438 births per woman (bpw), a 0.41% decline from 2020.[2] This rate is approximately half of what it was in 1950 (4.700 bpw), and more economically developed countries have even lower rates. The fertility rate in Europe is relatively low, with no country above 2.000 bpw, and has declined further in recent years.[1] The Americas have also seen widespread declines in fertility, with a regional fertility rate that is currently below replacement level at 1.900 bpw, ranging from 1.700 in North America to 2.200 in Central America.[3]

According to the American Pregnancy Association, male infertility accounts for 30% of infertility cases. Moreover, since the female literacy rate is increasing, females are more frequently opting to plan a family later in life due to their career prospects, which in turn makes females more dependent on fertility treatments as fertility decreases with age.[1]

According to a retrospective study conducted by Pi et al.[4] on the widespread application of assisted reproductive technologies (ART), the incidence of heterotopic pregnancy (HP) has increased. HP is a rare complication of pregnancy, in which both extrauterine and intrauterine gestation occur simultaneously.[5,6] The reported incidence is 0.6–2.5 out of 10,000 pregnancies.[5] The presence of an intrauterine pregnancy (IP) does not rule out the presence of a coexisting ectopic pregnancy (EP). Duverney was the first to report the occurrence of an HP in 1708, after finding an IP during the autopsy of a female who had died from a ruptured EP.[7] Devoe and Pratt[8] reported a heterotopic rate of 1 in 30,000 pregnancies, an estimate based on a theoretical calculation made in 1948. However, the incidence increases to 1–3 in every 100 pregnancies for pregnancies that follow ARTs, such as *in vitro* fertilisation (IVF) and gamete intra-fallopian transfer, or patients with a history of pelvic inflammatory disease (PID).[9]

In this article, the authors present a case of HP after 6 weeks and 3 days of gestation, diagnosed by transvaginal ultrasound (TVUS), which was managed with emergent laparoscopy. The IP course was uneventful, with the delivery of a healthy baby girl at term by caesarean section. This case study will serve as an example to increase awareness on HP early diagnosis and prompt management to improve maternal and fetal outcomes.

MATERIALS AND METHODS

A literature search was carried out to evaluate the occurrence of HP after assisted reproduction therapy, and a total of 31 original articles were included in the analysis. The authors investigated the risk factors, clinical presentation, diagnostic evaluation, treatment, and follow-up recommendations for each case. The authors' case report was produced on an HP that occurred after IVF and embryo transfer (ET) procedure in a patient post-unilateral salpingectomy, as an increased awareness of this rare life-threatening condition among practitioners is essential.

CASE PRESENTATION

A 31-year-old female (gravida: 2; para: 0; abortion: 0) presented at the authors' fertility clinic with mild pelvic discomfort and a good general condition otherwise. The patient had previously had a left salpingectomy due to an EP in her medical history, but no other medical conditions were reported. She had been trying to conceive for 3 years before she presented to the clinic and was diagnosed with infertility due to male factor. She underwent an intracytoplasmic sperm injection-IVF procedure and ET 6 weeks and 3 days before the presentation. This was an IVF fresh cycle attempt after previously failing an IVF fresh cycle and one frozen ET.

During the current cycle, the patient had two embryos transferred in Day 3('cleavage stage'). The *human chorionic gonadotropin* (hCG) test was done to check for the hormone hCG in her blood, 2 weeks after the ET procedure that resulted present. As per protocol, the patient was scheduled for follow up at the IVF clinic and to have her first ultrasound (US) scan performed at 7 weeks pregnancy. However, the patient presented at the urgent care clinic 4 days earlier than scheduled as she was experiencing mild discomfort in her right lower quadrant. The obstetrician/gynaecologist on duty performed a US scan, and informed the patient that she had a single vital IP, and sent her home. The next day, being in mild but consistent discomfort, the patient presented to the IVF clinic. A TVUS scan revealed two viable embryos with a crown rump length of 4 mm, corresponding to 6 weeks and 1 day of gestation, and detected minimal free abdominal fluid in the pouch of Douglas (Figure 1).

One intrauterine embryo was identified, and a second embryo was implanted in the right fallopian tube. Doppler ultrasonography revealed that both intrauterine and extrauterine fetuses were viable (Figures 2 and 3). The patient was stable and reported mild vaginal bleeding/spotting and right pelvic discomfort, rated 3 out of 10, which had no radiation and was constant and dull. The patient had a history of left salpingectomy 2 years prior to presentation, but no other history of PID, endometriosis, or trauma. She was found to have a haemoglobin level of 11.2 g/dL, haematocrit value of 27%, and a leukocyte count of 16,000/mm^3, and her renal and liver function tests and coagulation parameters were within normal limits. The patient was transferred to the operating room urgently and underwent a laparoscopic procedure. The EP was removed, and the IP made it safely to term delivery. The patient delivered her child at 39 weeks of pregnancy via caesarean section.

As HPs are a rare occurrence in the daily experience of general obstetricians gynaecologists, they are often hard to recognise. This case was a near miss that would have had fatal consequences to the intrauterine embryo and the mother if not diagnosed promptly.

DISCUSSION

Risk Factors

HP, defined as the simultaneous occurrence of IPs and EPs, is a potentially life-threatening and frequently misdiagnosed condition.[10] The most common risk factors for EP include PID, intrauterine devices, adhesions, a previous history of EP, ARTs, and ovarian hyperstimulation syndrome.[11,12] Pi et al.[4] also reported that tubal infertility and pelvic adhesion increase the risk of HP among patients undergoing IVF treatment; however, a cohort study conducted by Xiao et al.[13] reported no significant difference in the incidence of HP in fresh IVF cycles versus frozen-thawed cycles. In a retrospective study by Lv et al.,[14] the authors found that a history of EP and previous tubal surgery may increase the risk of HP. In addition, low levels of serum hCG and oestrogen in the patient on Day 14 after ET could indicate the incidence of HP. Jeon et al.[11] reported that patients undergoing IVF and ET with a history of EP, abortion, and ovarian hyperstimulation syndrome may be at increased risk for HPs compared with the control group of other IVF patients. Liu et al.[15] indicated that the transfer of two or more embryos in an IVF procedure, low β-hCG and progesterone levels on Day 14 after ET, and vaginal bleeding should be considered as predictors for HPs.

A retrospective study conducted by Luo et al.[16] on 1,476 pregnancies following IVF-ET procedures reported 12 (0.81%) such HP cases. In this paper, they attributed the increase in HP cases to the increased incidence of genital infections and widespread use of ovulation

Figure 1: A transvaginal ultrasound displaying the heterotopic pregnancy outlined in this case study.

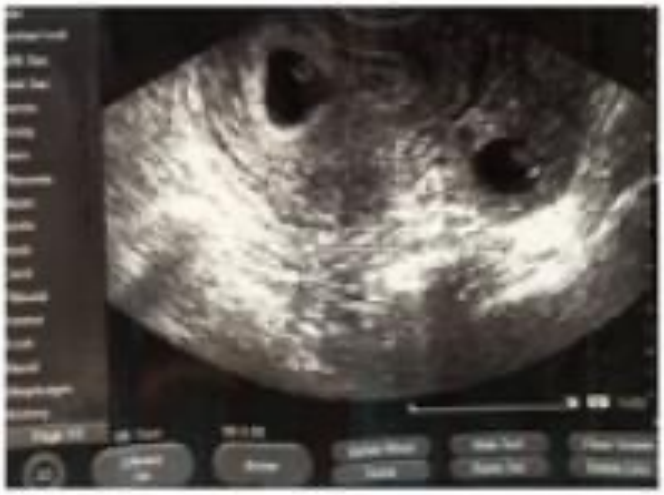


Figure 2: A Doppler ultrasound of a viable extrauterine pregnancy.

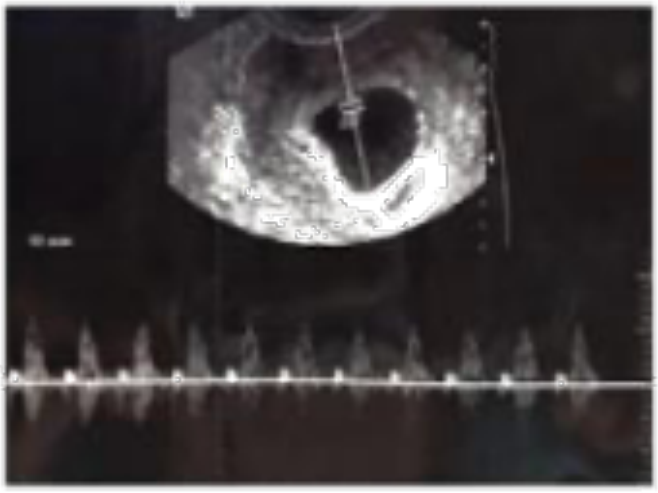
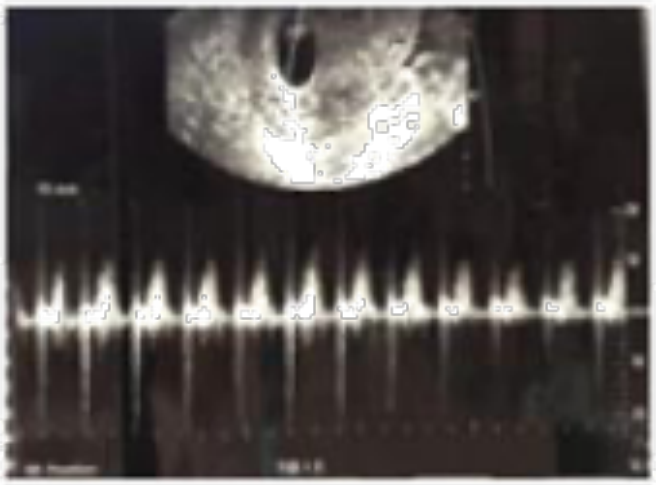


Figure 3: A Doppler ultrasound of a viable intrauterine pregnancy.



induction agents.[16] Exogenous hormones, uterine contractions that occur during the transfer of the fertilised embryo, inadvertent introduction of the embryo directly into the fallopian tube, and the volume and number of embryos transferred are all risk factors that are specifically encountered in ART.[16,17] Risk factors for HPs, as noted by Nabi et al.,[10] also include a personal history of tubal diseases and the use of ART. Furthermore, Soares et al.[18] observed that the risk factors for an HP closely mirror those of an EP, and, based on a meta-analysis performed by Ankum et al.,[19] these were found to be: a history of EP, tubal surgery, or pathology; *in utero* diethylstilbestrol exposure; and previous genital infections, including PID, chlamydia, and gonorrhoea.

Gergolet et al.[20] described a case of HP that occurred after a single embryo and blastocyst transfer. In this example, intercourse took place on the day of hCG administration to a patient, where an oocyte was not collected during the oocyte retrieval procedure, which resulted in an HP in Week 11 of pregnancy.

As with EPs, the most common ectopic site for HPs is the fallopian tube; implantation in the cervix, ovary, abdomen, and pelvis is extremely rare.[10] Approximately 60–70% of HPs result in live births, with similar outcomes to singleton pregnancies.[10] Luo et al.[16] reported this figure to be 66.7% at the Reproductive Centre in Guangdong Women and Children's Hospital, China. The high mortality rate of intrauterine gestation may be attributed to delays in diagnosis, but, if left to progress, this condition could also lead to maternal mortality.[10]

Clinical Presentation

In a study conducted by Reece et al.[21] that reviewed 589 cases of HPs globally, abdominal pain was reported to be the most common presenting symptom. Other signs and symptoms most commonly found in support of a presumptive diagnosis of an HP included the presence of an adnexal mass, peritoneal irritation, and an enlarged uterus.[21] However, these signs and symptoms are unspecific, and may be found in other gynaecological and non-gynaecological conditions such as a miscarriage, EP, IP with adnexal torsion, appendicitis, a bowel obstruction, cholecystitis, or pancreatitis.[18,] However, it is rare for an HP to present with vaginal bleeding.[21] Nabi et al.[10] reported the non-specific presence of abdominal pain in 72% of HPs and vaginal bleed in 54% of HPs. It was reported by Soares et al.[18] that, as a consequence, most diagnoses for HPs are delayed, and are only made after rupture of the EP. A high index of suspicion is, therefore, required for an accurate and timely diagnosis in order to reduce maternal morbidity and mortality, which currently stands at 1 in 200,000 live births.[22] To complicate matters further, up to 24% of cases may present asymptomatically.[10] In a retrospective review by Lyu et al.[23] on 55 patients with HPs, it was found that only 29.1% of patients presented with abdominal pain, while 34.5% of patients were asymptomatic prior to diagnosis.

Diagnostic Techniques

A review by Talbot et al.,[24] which looked at cases published prior to 2010, noted the increased role of US scans in the definitive diagnosis of HPs. Indeed, TVUS remains the gold standard for the diagnosis of an HP.[18] However, as the detection of an IP often leads to the mistaken exclusion of a concomitant EP, it is recommended that careful adnexal US assessment be carried out to mitigate the risk of missing an HP.[18] In the study by Lyu et al.,[23] the authors suggested that routine TVUS at Day 27 following ET could facilitate the diagnosis of an HP, leading to fewer missed diagnoses. Current National Institute for Health and Care Excellence (NICE) guidelines also outline the importance of scanning the uterus and both adnexae when performing a TVUS early in the pregnancy to check for an HP.[22]

Separately, studies by Yoshigi et al.[25] and Si et al.[26] that aimed to determine the role of MRI in the early diagnosis of an EP reported that the use of T2-weighted imaging was highly accurate in the detection and diagnosis of EPs. Masselli et al.[27] also retrospectively reviewed 150 patients with a suspected diagnosis of EP, and analysed the clinical, US, and MRI features of 15 unusual cases. The authors reported that diffusion- and fat saturation T1-weighted images were the most accurate for the detection of EPs, picking up 100.0% and 93.3% of the 15 cases, respectively.[27] As such, the authors concluded that the use of MRI for the early diagnosis of early EPs should be considered, following negative US findings in some highly suspicious cases.[27]

Management

Treatment methods for EPs currently include medical management with methotrexate, surgical management with salpingostomy or salpingectomy, and expectant management.[28] It appears that the surgical treatment of HPs does not appear to affect the rates of first trimester fetal loss or live birth.[12] However, the use of methotrexate in HPs should be avoided, given its teratogenic effects on the viable IP.[12,29] Li et al.[30] performed a prospective study on 64 patients diagnosed with HPs between January 2003 and June 2014, of which 12 patients were excluded on grounds of a non-viable IP. The remaining 52 patients either received expectant management (n=20), surgical management (n=27), or transabdominal US-guided transvaginal aspiration of the ectopic gestational embryo (n=5).[30] The surgical management group included those who underwent an emergency and elective laparotomy and laparoscopy.[30] Of the 20 patients managed expectantly, six eventually converted to surgical treatment. Of the 27 patients managed surgically, there were four abortions and one stillbirth. Transabdominal US-guided transvaginal aspiration resulted in 100% live births without the need for secondary conversion to any other treatment methods.[30] This led the authors to conclude that transabdominal US-guided transvaginal aspiration gave rise to the lowest abortion rate and best maternal outcome, while surgical management led to the highest abortion rates and expectant management resulted in the worst maternal outcomes.[30] However, the degree of difficulty in utilising transabdominal US-guided transvaginal aspiration of the ectopic embryo depends on the location of the ectopic gestational sac, and should be attempted only when this can be clearly visualised.[30]

Yu et al.[31] reported that surgery is still the most frequently chosen form of treatment for the management of an HP, and that it involves a salpingectomy in most cases, although the selected method ultimately depends on the actual clinical condition of the patient. It is further recommended by Ciebiera et al.[32] that the manipulation of the uterus be kept to a minimum during surgery in order to protect the IP from potential complications.

Recommendations

The patient study outlined in this article is a classic case, presenting with the typical risk factors associated with a left salpingectomy and the use of ART, as well as the most commonly encountered complaint of mild abdominal discomfort. Despite this, the general obstetrician/gynaecologist attending physician was unable to arrive at an accurate diagnosis. Given the difficulty encountered in diagnosing HPs, particularly in patients who do not present classically or in whom physicians do not have a high degree of suspicion for, and that ART and the incidence of HPs have been on the rise in recent years, it appears advisable to recommend that physicians perform a TVUS on ART patients at 27 days following the ET process, as reported by Lyu et al.[23] Ultimately, the patient underwent an emergency laparoscopy to remove the ectopic gestational embryo, allowing for the successful carriage of the IP to term.

From this case, we can see that it is indeed challenging to diagnose an HP in view of the non-specific clinical and laboratory findings surrounding the condition. It is, however, essential to highlight the importance of correct diagnosis and prompt treatment, as failure to do so poses a threat to both the viable and the developing fetus, as well as to the mother. Every red flag should, therefore, be carefully evaluated and consulted by a fertility team, if required.[33]

CONCLUSION

HPs are very difficult to diagnose because of the rarity of their occurrence in everyday practice. An HP may occur in any woman of reproductive age, especially in induced ovulation or IVF cases. In patients undergoing IVF treatment, additional attention is recommended during the first US scan, especially in females where more than one embryo is transferred. Careful scanning of the uterus and appendages is necessary in all females of reproductive age with clinical symptoms. Routine TVUS could facilitate the diagnosis of HP, and timely diagnosis can change the outcome of the pregnancy and decrease complications for the patient. Laparoscopic intervention is the treatment of choice in haemodynamically stable patients, as a laparoscopy can safely achieve the removal of ectopic implantation with minimal trauma

whilst avoiding intraperitoneal haemorrhage and complications with the concomitant IP. A laparotomy has more associated complications, but these are indicated by severe intra-abdominal bleeding or haemorrhagic shock.

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Immediate Perinatal Outcome of Mothers with Maternal Near-Miss at Moi Teaching and Referral Hospital, Eldoret, Kenya

Authors:	*David Nding'ori, Winfred Mwangi, Pallavi Mishra, Jack Odunga Department of Reproductive Health, Moi University School of Medicine, Eldoret, Kenya *Correspondence to ndingoridavid@yahoo.com

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Abstract

Objective: To compare the immediate perinatal outcome of females with maternal near-miss (MNM) morbidity and those without near-miss morbidity in Moi Teaching and Referral Hospital (MTRH), Eldoret, Kenya.

Methods: A case-control study was conducted at MTRH. Near-miss cases were identified using World Health Organization (WHO) criteria for near-miss. A consecutive sampling technique was applied to obtain a total of 45 cases and 225 controls. Determinants of near-miss were assessed using a binary logistic regression model. Perinatal outcomes were compared between the near-miss and control.

Results: Severe maternal outcome ratio was 11.4 per 1,000 live births, the MNM ratio was 10.4 per 1,000 live births (95% confidence interval [CI]:7.6–13.9), and MNM mortality ratio was 11.2. The most common condition associated with near-miss was haematological or coagulation dysfunction (64%). Hypertension was the leading underlying cause of near-miss (35%). Factors associated with reduced odds of near-miss were employment (odds ratio: 0.12; 95% CI: 0.03–0.42) and awareness of danger signs (odds ratio: 0.41; 95% CI: 0.19–0.91). Most babies of the near-miss cases were born alive (76%), with median appearance, pulse, grimace, activity, respiration (Apgar) score of 9/10; stillbirth rate was 22%; and median birth weight was 2,700 g. This was similar to the control group with live birth at 77%, a median Apgar score of 9/10, and stillbirth of 23%. There was no statistically significant difference in perinatal outcome between cases and control.

Conclusion: MNM indicators are comparable to the world. The determinants of near-miss are unemployment and lack of awareness of danger signs. There was no difference in perinatal outcome between the cases and control.

Key Points

1. The identification of maternal near-miss is important for preventing complications that lead to death and for highlighting opportunities to avoid similar cases in the future.

2. This case-control study examined the maternal near-miss indicators among pregnant patients at a Kenyan Hospital, finding that the determinants of near-miss were unemployment and lack of awareness of danger signs.

3. Education on danger signs should be encouraged, and peripheral facilities should be equipped with theatre and personnel to reduce referral and offer timely intervention.

INTRODUCTION

Improving maternal health has been a major concern worldwide. This is evident by maternal health being included in the Millennium Development Goals, with the goal of reducing maternal mortality by three-quarters by 2015, and now the Sustainable Development Goals, with targets set to reduce the global maternal mortality ratio to less than 70 per 100,000 live births. It further states that no country should have maternal mortality above 140 per 100,000 live births by 2030.[1]

Progress on reduction of maternal mortality has been slow. Globally, the estimated maternal mortality of 2013 was 289,000, with sub-Saharan Africa accounting for 62% of this.[2] Although this was a 45% reduction from 1990, this has been very far from what was set to be achieved in the Millennium Development Goals. Most of the countries, including Kenya, did not achieve the Millennium Development Goals, which expired at the end of 2015.

Reduction in maternal mortality has traditionally been used as a critical measure of maternal health, but this represents only a glimpse of the burden of maternal morbidity. For every maternal death, there are close to 100 females with severe maternal morbidity (SMM) referred to as maternal near-miss (MNM).[3] Hence, relying solely on maternal mortality to assess maternal health overlooks the pregnancy continuum from normal to death. On this continuum, pregnancy, labour, or the puerperium may be classified as uncomplicated, complicated, severely complicated, life-threatening, or fatal.[4] In life-threatening pregnancy-related complications, the female has one of two severe maternal outcomes: they may die (maternal deaths) or narrowly escape death (MNM cases).

MNM is defined as a female who nearly died but survived a complication that occurred during pregnancy, childbirth, or within 42 days of termination of pregnancy.[5] Theoretically, these females are considered near-miss retrospectively when they survive organ dysfunction. Females who undergo a MNM have many common characteristics with those who die from the complication.[5] Similar to the cases of maternal mortality, MNM cases are preventable. Thus, the identification of MNM is important for preventing complications that lead to death and for highlighting ways and opportunities to avoid similar cases in the future.

MNM approach is being used to describe local patterns of maternal mortality and morbidity, strengths and weaknesses in the referral system, and the use of clinical and other healthcare interventions. Despite its wide application, there were challenges with its use mainly due to the absence of universal criteria for the identification of cases. In 2009, a criterion for MNM identification was developed by the World Health Organization (WHO) working group for maternal health so as to standardise the detection of MNM cases. This is a two-step process. Firstly, maternal cases with potentially life-threatening conditions such as SMM, which may or may not be near-miss cases (e.g., specific complications such as severe pre-eclampsia and/or critical interventions such as blood transfusion), are identified. Secondly, identification of near-miss

cases based on organ system dysfunction and organ dysfunction proxies, including clinical, laboratory, and management criteria.[6]

In Kenya, MNM morbidity review is considered one of the many strategies to tackle high maternal mortality. The national maternal and perinatal death surveillance and response (MPDSR) committee recommend a review of MNM cases in order to reduce the maternal mortality rate to less than 200 per 100,000 live births by 2030.

Problem Statement

Inquiries into maternal healthcare have for a long time used maternal death as the starting point of investigations. Death is the worst maternal adverse event in pregnancy and viewing the circumstances around the death may reveal some avoidable health factors. However, despite the high maternal mortality ratios, in many countries with resource-poor settings, maternal deaths are rare in absolute numbers per centre. This does not allow detailed quantification of the associated risk factors and determinants that are locally important. In the last 20 years, the idea of MNM has been explored in maternal health as an adjunct to maternal death confidential enquiries. Among other advantages, near-miss cases occur more frequently than maternal deaths and can directly inform on problems and obstacles that had to be overcome during the process of healthcare, providing more robust conclusions and rapid reporting on maternal care issues.[7]

Study Objective

To determine the MNM indicators among females who are pregnant seeking healthcare at Moi Teaching and Referral Hospital (MTRH), Eldoret, Kenya.

METHODS

Study Setting

The study was carried out at the maternity wing, gynaecology ward, cardiac unit, intensive care unit, high dependency unit, and renal unit of MTRH.

Study Design

This was a case-control study of females who nearly died but survived a complication that occurred during pregnancy, childbirth, or within 42 days of termination of pregnancy (near-miss). The control group was females with similar conditions causing a near-miss but does not fulfill the near-miss criteria. For each case of near-miss, four matched controls were selected. The age, gestation age of pregnancy, parity, and condition causing near-miss were considered during the match selection.

Study Population

The study population was females who were pregnant, those within 42 days of termination of pregnancy, and babies delivered to these mothers seeking healthcare at the MTRH.

Sample Size

The exposure rate among the controls was 47%. Hence, with 45 cases and 180 controls (based on a ratio of four controls for every case), a total of 225 participants were recruited for the study.

The exposure rates were calculated from records of prior cases in the hospital records. The indirect causes of near-miss were deduced from the three delays model, which includes decision making, reaching the facility, and receiving adequate care.

Sampling Technique and Study Procedure

The sample population included all females presenting to the hospital seeking healthcare during pregnancy, labour, delivery, and/or within 42 days after delivery or termination of pregnancy. Once any of these females were discharged from the maternity wards, they were screened for the presence of any SMM or potential life-threatening and a severe maternal outcome like MNM. The identification of SMM was based on diagnostic categories such as obstetrical haemorrhage, hypertensive disorders, sepsis or severe systemic infection, uterine rupture, early pregnancy complications, and/or other indirect causes. The females who had SMM were then screened as per WHO criteria for near-miss and those who qualified were included in the study.

Once a near-miss was identified, matched controls were then selected from the population of females with potentially life-threatening conditions in the wards. In order to reduce bias as a result of chance, four controls per near-miss were included.

Data Management and Statistical Analysis

Data was collected using a structured questionnaire. Data analysis was conducted using software for statistical computing called R.[8] Categorical variables, such as level of education, marital status, source of income, residence, and possession of health insurance, were summarised using frequencies and the corresponding percentages. Continuous variables, such as gestational age, and discrete variables, such as the number of pregnancies and number of births, were summarised using the mean and the corresponding standard deviation.

Comparison of categorical variables by cases and controls was performed using Fisher's exact test due to violation of Pearson's chi-squared assumptions for most categorical variables. Continuous and discrete variables were compared using two-sample Wilcoxon rank-sum test and independent samples t-test. The authors reported the associated p values.

Determinants of near-miss were assessed using a binary logistic regression model. Factors that were established to be associated with near-miss in the bivariate analysis were all included in the multivariate logistic regression model. The authors reported the odds ratios (OR) and the corresponding 95% confidence intervals (CI).

Ethical Considerations

Approval to conduct the study was sought and obtained from the institutional research and ethics committee. Permission to conduct the study was sought from MTRH. Informed consent from the participants and guardians for those less than 18 years. Consent was obtained for minors (under the age of 18 years).

RESULTS

The proportions of participants with a level of income more than 6,000 KSH were similar for the cases and the controls (11.1% versus 16.1%; p=0.491). The MNM cases were significantly less likely to have health insurance than the controls (15.6% versus 31.1%; p=0.042).

The female's reproductive and obstetrical information on the current pregnancy was collected. The median gestational age was similar for the cases and the controls: 38 weeks (interquartile range [IQR]: 36–38 weeks) versus 38 weeks (IQR: 36–39 weeks); p=0.461. There was no evidence from the data on the difference in the modes of delivery between the two groups (p>0.05). There was borderline significance in the proportion who attended the antenatal clinic between the cases and the controls (84.4% versus 93.9%; p=0.052).

A significantly lower proportion of the cases were aware of the danger signs of pregnancy compared with the controls (26.7% versus 48.3%; p=0.011). The proportion admitted to the hospital during the current pregnancy among the cases and among the controls were similar (26.7% versus 18.3%; p=0.216). Similarly, the proportion who had a birth plan among the cases and among the controls were also similar (51.1% versus 64.2%; p=0.124).

Female's Reproductive and Obstetrical Information on Current Pregnancy

Data on the past obstetric history was collected. The findings demonstrated that a significantly higher proportion of participants among the cases had an interpregnancy period of more than 3 years (46.7% versus 14.9%; p<0.001).

There was no difference in the proportion of participants who had a history of complications in the previous pregnancy among the cases and among the controls (53.3% versus 61.9%; p=0.414). However, among the participants who had a history of complications in pregnancy, the proportion of participants who had experienced stillbirths in the MNM cases was significantly higher than that observed among the controls (37.5% versus 7.2%; p=0.004).

History of chronic illnesses was reported by a higher proportion among the cases (35.6%) relative to the controls (29.1%); however, there was insufficient evidence to link the history of chronic illnesses with near-miss (p=0.468). The proportion of participants who reported specific chronic illnesses among the cases and among the controls was not statistically significantly different (p>0.05).

Overall, 77.5% of the children were born alive. This represented 76.3% among the cases, and 77.8% among the controls. The fresh and macerated stillbirths accounted for 22.5%, with 23.7% among the cases, and 24.2% among the controls. This was not statistically different between the groups (p=0.831).

The median birth weights were similar for the cases and the controls (median birth weight: 2,700 g [IQR: 2,000–3,050 g] versus 2,850 g [IQR: 2,600–3,100 g]; p=0.068). Similarly, the median Apgar scores were similar for the MNM cases and the controls (9.0 [IQR: 2.0–10.0] versus 9.0 [IQR: 7.8–10.0]; p=0.429).

The proportion of children admitted to the newborn unit among the cases and the controls (46.4% versus 44.8%; p>0.999), as well as the proportion of children alive and discharged or alive by Day 7 among the cases and the controls (85.7% versus 93.1%; p=0.249) were similar.

The results show that most of the participants who were diagnosed with near-miss presented with haematological or coagulation dysfunction (64.4%), followed by cardiovascular dysfunction (24.4%). The underlying cause of near-miss was mainly hypertensive disorders (35.6%), followed by medical/surgical diseases (28.9%).

DISCUSSION

Perinatal Outcome

There was no difference in birth weight, stillbirth, admission to the newborn unit, Apgar score, and condition on the discharge of the babies born between the cases and control.

This finding was similar to a study in Brazil that showed no difference in Apgar score at 5 minutes and birth weight. This finding was different from a study in Nigeria that found near-miss was associated with stillbirth, low birth weight, and postmature pregnancy.[9,10] This study was different from the authors' study in that it used unmatched controls, meaning their controls had normal pregnancy and hence could differentiate from the MNM cases. In the authors' study, the controls were matched with the cases hence similar underlying pathology, which could have contributed to a similar outcome.

It was noted that the active phase of labour taking more than 6 hours was associated with near-miss compared with controls. Most of the near-miss came to the hospital as referrals. The third delay, which is referral from one facility to the final facility, of more than 2 hours was found to be significantly higher in the near-miss group relative to the control. The most common reason for referral was the lack of personnel. It was also found that those who made decisions within 30 minutes of symptom onset were more likely to be near-miss.

Although there is no near-miss study that looks at the duration of labour as a risk factor, prolonged labour is associated with several adverse maternal outcomes (e.g., postpartum haemorrhage, uterine rapture, puerperal sepsis, and caesarian section), and these conditions could cause near-miss.[11]

Several studies have linked near-miss to delays. Most attribute to all the delays, namely delay in decision making, delay in arriving at the hospital, and delay in attaining medical care due to either lack of personnel or poorly equipped hospitals leading to referral to a more equipped hospital.[12-14] Most hospitals in developing countries are under-resourced, leading to delays in initiating treatment and offering timely referrals.[15] Seeking care from a facility that is ill-equipped to give emergency obstetric care contributes to significant delay even after reaching the health facility. These factors were reported as significant contributors of delay in several studies.[16] As a matter of fact, these non-functional health facilities are physically accessible but act as physical obstacles for females who are pregnant in accessing a functioning health facility in time. From the authors' study, third delay was associated with near-miss, especially among those who sought treatment in a health facility with no personnel or

Table 1: Perinatal outcomes.

		Control	Cases	
Variable	**N**	**n (%) or median (IQR)**		**p**
Fetal status				
Alive	191	119 (77.8%)	29 (76.3%)	0.831
Fresh stillbirth	191	24 (15.7%)	7 (18.4%)	0.632
Macerated stillbirth	191	10 (6.5%)	2 (5.3%)	>0.999
Birth weight	180	2,850 g (2,600–3,100 g)	2,700 g (2,000–3,050 g)	0.068
Range (min–max)	N/A	980–3,900 g	1,100–3,720 g	N/A
Apgar score at 5 minutes	144	9.0 (7.8–10.0)	9.0 (2.0–10.0)	0.429
Range (min–max)	N/A	0–10	0–10	N/A
Baby admitted to NBU	144	5.0 (44.8%)	13.0 (46.4%)	>0.999
Baby alive on discharge or at Day 7 of life	144	108.0 (93.1%)	24.0 (85.7%)	0.249

IQR: interquartile range; max: maximum; min: minimum; N/A: not applicable; NBU: newborn units.

lack of facility to handle the emergency. Those who took more than 2 hours to reach the final destination were associated with near-miss. This is true because almost all government facilities in Uasin Gishu County, Eldoret, Kenya, can only offer basic obstetric care, and this leads to delays in offering care to the females at risk of developing a near-miss.

Most of the patients with a near-miss were referred to or came to the hospital with complications already. Only two of the complications developed in the hospital. This is in keeping with another study in a tertiary hospital in this region, which showed that most onsets of near-miss complications happen in referring facilities.[15] The complexity of care and treatment provided to patients in the obstetric ward ranges from basic to intensive care and thus the level of health facilities is different too.[17]

Causes of Near-Miss

The results show that most of the participants who were diagnosed with near-miss presented with haematological or coagulation dysfunction (64.4%), cardiovascular dysfunction (24.4%), neurological dysfunction (15%), renal dysfunction (13%), and hepatic and respiratory dysfunction (8%). The underlying cause of near-miss was mainly hypertensive disorders (35.6%), followed by medical/surgical diseases (28.9%), and obstetric haemorrhage (17%).

These findings are similar to a study in Malaysia,[18] which found haematological or coagulation dysfunction to be the most common cause of organ dysfunction. The underlying cause of near-miss was hypertensive disorder, and this is similar to studies carried out in the region.[15,19] The similarity is also observed in complications caused by medical/surgical diseases, especially

sepsis.[19] Obstetric haemorrhage came third, which is also similar to other studies. It could be due to the widespread use of protocols for the management of postpartum haemorrhage even at the lower-level facilities. Direct causes of near-miss were different compared with a study in Kenyatta National Hospital, Nairobi, Kenya, which found that haemorrhage and hypertension were the most common diseases causing near-miss.[20] The difference could be due to changing times. In the last decade, there has been a lot of advocacy to reduce postpartum haemorrhage by use of uterotonic drugs. Indeed, a recent study showed up to 95% of delivering females received uterotonic drugs as management of the third stage, which could explain the reduction.[21]

CONCLUSION

There was no difference in perinatal outcome between near-miss cases and the controls.

RECOMMENDATIONS

- Health education should be encouraged on the danger signs, during antenatal clinic visits, as many cases of near-miss demonstrated a lack of awareness of danger signs.
- Equipping the peripheral facilities with theatre and personnel to reduce referral and offer timely intervention. The most common reason for referring was lack of personnel and expertise.

STUDY LIMITATIONS

This study is a hospital-based study and its findings may not be generalised. MTRH is the only government facility offering comprehensive obstetric care within Uasin Gishu County. Therefore, the large concentration of pregnant females with previous comorbidities and obstetric complications might have overestimated the indicators. This was a case-control study, hence could not be used to estimate the incidence of near-miss.

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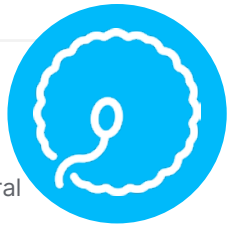
Female's Experience with Post-placental Intrauterine Contraceptive Device Use in a Tertiary Care Centre in Pakistan



Authors:	*Zahra Safdar,[1] Sumaira Fatima Sabir,[2] Sumaira Zaib,[2] Saleeha Abid[2] 1. Central Park Medical College, Lahore, Pakistan 2. Department of Obstetrics and Gynecology, Lahore General Hospital, Pakistan *Correspondence to zsafdar2@gmail.com

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Abstract

Background: Insertion of a post-placental intrauterine contraceptive device (PPIUCD) has been recommended by the World Health Organization (WHO) as one of the safe and effective methods of temporary contraception. In the immediate post-delivery period, females are highly driven and in need of an effective method for contraception so that child-rearing can be easy without unintended pregnancy.

Objective: To assess the outcome of females accepting PPIUCD in terms of insertion, complications, and discontinuation rate at 6 weeks and 6 months.

Methods: Retrospective, cross-sectional study conducted at the Department of Obstetrics and Gynecology, Lahore General Hospital, Pakistan, from May 2015 to August 2019. The data were collected and analysed after approval from the hospital ethical committee and were retrieved from the maternal and neonatal child health programme. Record of clinical visit and telephonic survey for miscellaneous complaints at 6 weeks and 6 months were evaluated from the medical records department and relevant data were extracted. The data were analysed using the IBM Statistical Package for the Social Sciences (SPSS® Statistics) V21.0 software (International Business Machines Corporation [IBM], New York, USA) and the results were expressed in descriptive statistics in frequencies and percentages.

Results: Total live births during the study period were 43,065. PPIUCD was inserted in 5,275 females (12.24%). Only 13% presented for clinical follow-up; 87% had a telephonic conversation. Of these, 33% were advised to have a clinical visit; 83% reported no complaints. Reassurance was needed in 11% and threads were trimmed in 2%. Problems reported were displaced intrauterine contraceptive device (IUCD;

3.3%); spontaneous expulsion (24.0%); vaginal infections (4.7%); missing strings (3.0%); cramping (25.0%); dyspareunia (6.0%); spotting (52.0%); and vaginal discharge (16.0%). Ultrasound was advised (3.0%), as were symptomatic treatment (14.0%) and antibiotics and IUCD removal (0.7%). IUCD was discontinued by 6.7% of the females for various reasons: family pressure (0.3%); wanting a further child (0.7%); and opting for another method of family planning (14.0%). IUCD was removed and reinserted in 4.9%.

Conclusion: PPIUCD has a low turnover rate of follow-up but has high compliance with devices and a low complication rate; however, complications can be reduced by improving patient selection and clinical follow-up. It provides an excellent window of opportunity for providing effective long-term contraception to the patients who need it the most.

Key Points

1. Consecutive pregnancies spaced less than 24 months apart carry a higher risk of premature labour, miscarriage, low birth weight, post-partum haemorrhage, and foetal loss. Spacing out pregnancies by 2 years could avert 10% of child and one-third of maternal deaths.

2. This retrospective, cross-sectional study analysed data on the clinical follow up of the insertion of post-placental intrauterine contraceptive devices in 5,275 females in Lahore General Hospital, Pakistan.

3. Misconceptions around the use of intrauterine devices at community level remain an issue, however, multiple studies have indicated high continuation and satisfaction rates.

INTRODUCTION

Pakistan is referred to as sixth most populated country in the world. In 37% of females, the gap between two births is almost less than 24 months. Contraceptive use is highest in those aged between 34–36 years. Between various reversible family planning methods, couples usually use either condoms or injectable contraceptives. The female sterilisation method is used as a permanent method. Recently, family planning resources have reported 64.4% unmet needs, which is leading to low contraceptive prevalence in Pakistan.[1]

One-third of maternal deaths and 10% of child mortality can be averted by family planning, which happens when pregnancies are spaced by couples for more than 2 years apart.[2] Small gaps between the births are linked with higher child mortality and higher maternal morbidity and mortality.[3] With respect to the previous birth, pregnancies taking place within the time period of 24 months show a higher risk of premature labour, miscarriages, low birth weight babies, post-partum haemorrhage, and fetal loss. From 2005–2006, India's National Family Health Survey (NFHS) confirmed that 61% of births were spaced less than 3 years[4] and that 22% of married females had an unmet need for family planning. In India, 65% of females have an unmet need for contraceptives in the first year post-partum, for family planning.[5] Post-placental intrauterine contraceptive device (PPIUCD) insertion is a convenient, safe, and effective method for post-partum contraception.[6] After the removal of the placenta, immediate intrauterine device (IUD) insertion has not been associated with uterine subinvolution, increased risk of uterine perforation, post-partum bleeding, or increased infection.[7] It was observed that expulsion rate was higher in immediate PPIUD insertion when compared with insertion at an interval of 4–8 weeks.

IUDs are a safe, highly effective, and long-acting form of contraception. Since the 1960s, the concept of IUD insertion as a post-partum IUD has become acceptable and has been investigated worldwide. Where females have access to medical care, IUD delivery provides a significant opportunity for the contraception

need. Post-partum family planning is accepted to prevent closely spaced and unintended pregnancies in the first 12 months following childbirth.[7] Effective contraceptive methods are a major need in order to prevent an unplanned pregnancy within a short interval.[8]

According to World Health Organization (WHO) Medical Eligibility Criteria, the insertion of an intrauterine contraceptive device (IUCD) can be done within 48 hours post-partum.[9] According to the availability of options, the copper T (CuT) 380A IUD, which has a multi-year cost, is considered to be the most cost-effective contraceptive option available. The CuT 380A IUD is a non-hormonal and effective method that can be used by all females, regardless of their breastfeeding status.[10] IUCD is a long-acting reversible method of contraception with 12 million current users worldwide.

METHODOLOGY

This paper outlines a retrospective, cross-sectional study conducted in the Department of Obstetrics and Gynecology, Lahore General Hospital, Pakistan, from May 2015 to August 2019. The study was conducted after approval from the hospital ethical committee. The total live births during the study period were 43,065; PPIUCD was inserted in 5,275 females (12.24%). The comparative data of patients' clinical follow-ups, visits, and problems were assessed using the IBM Statistical Package for the Social Sciences (SPSS® Statistics) V21.0 software (International Business Machines Corporation [IBM], New York, USA), and the results were expressed in descriptive statistics in frequencies and percentages.

RESULT AND DISCUSSION

This retrospective study of PPIUCD insertion was conducted from May 2015 to August 2019 in a tertiary centre in Pakistan. PPIUCD insertions in 5,275 females showed that a large number of females agreed to space out their next pregnancy with the choice of insertion of an IUCD immediately after removal of the placenta and within 30 minutes of delivery. To signify the use of PPIUCD, counselling of the couple should occur in the antenatal period and before delivery, which can be very effective in having them leave the hospital already protected against unplanned pregnancy. After the removal of the placenta, immediate insertion of an IUD satisfies the basic requirements of contraceptive methods.[11] Other females accepted this contraceptive method because they did not want any more children. The complications and issues associated with this method were also very low. To limit future childbearing, females mostly accepted the method and indicated the PPIUCD as their long-acting reversible contraceptive method.[12]

The majority of females (as high as 83%) reported accepting PPIUCD as a method of contraception because it is long-acting (Table 1), while many females (22%) accepted the PPIUCD insertion due to its free-of-cost service. Significantly, 50% of the females had heard of the IUCD before they were consulted at the facility, and only 7% had used one before they visited the facility, hence the vast majority of clients had accepted the PPIUCD service.[13] During this study, it was also observed that nearly all females were satisfied at the time of IUCD insertion, and it was reported that they were happy with IUCD at 6 weeks following insertion of the device.

In the present study, out of the females who discontinued the use of PPIUCD: 1% of females faced family pressure to discontinue the method; 4% wanted a future child, so discontinued the use of this method; and 70% of females opted for another method of contraception. Some users experienced side effects and others reported the removal and reinsertion of the IUCD due to other reasons (0.25%). The common issues (Figure 1) associated with this method of contraception were noted as being: displacement of IUCD (3.3%); vaginal infections (4.7%); missing strings (3.0%); cramping (25.0%); dyspareunia (6.0%); vaginal discharge (62.0%); and vaginal spotting and bleeding (52.0%). About 3.8% of females had removed their PPIUCD within the first 6 weeks of insertion. For minor issues, some of the users were advised symptomatic treatment (14.0%), some had to undergo a pelvic ultrasound

(3.0%), while others had infections for which they needed antibiotics and IUCD removal (0.7%) (Figure 2).

There is a room for strengthening PPIUCD counselling services regarding complications, like uterine perforation and other common side effects that arise from IUCD use, that lead to the discontinuation of this suitable method of contraception.[14,15] Awareness of the common issues associated with the use of the PPIUCD in the antenatal period prior to insertion would be expected to have a positive effect on the continuation of the use of this method. Counselling about minor issues and ease of treatment would be associated with a reduction or discontinuation of the PPIUCD use. Doubts of users should be addressed before its insertion, just after delivery.[16]

In the authors' case, the continuation rate of use of PPIUCD was observed as >75 per 100 users for CuT 380 devices, showing the acceptability of post-placental insertion of IUCD as an effective and safe method of long-term contraception.

By educating the target population, the attitude towards traditional methods has changed, as have concerns about their safety due to developing innovations in family planning practices. Recent studies that were done on the post-partum contraceptives used among adolescent mothers confirmed a lower risk of rapid repeat pregnancy in those using long-acting reversible contraceptive methods than in those who adopted short methods, like oral contraceptive pills.[17]

A detailed study of immediate PPIUD indicated that its insertion neither increased the risk of infection nor the amount of bleeding. In various reports, CuT IUDs had a higher expulsion rate when inserted after delivery following the removal of the placenta compared with delayed insertion.[18]

Many females are accepting this method to limit them bearing future children, showing the important place that PPIUCDs hold as a long-acting reversible contraceptive method. In terms of policy implementation, the family planning programme has transformed to assist in fertility control and has been stressing the cafeteria approach. In the cafeteria approach, various contraceptive methods are offered to clients, from which they can select their contraception based on their age, profession, parity, social class, and duration of birth spacing required.[19]

Effective contraception after childbirth assists in the health improvement of mothers and infants, whilst giving mothers time to bond with their babies. Rates of major maternal complications like third-trimester bleeding, anaemia, puerperal endometritis, and death all decrease due to the longer birth interval, thereby improving the quality of life of both mother and baby. In breastfeeding females, contraception with PPIUD is highly effective and does not impair lactation. Lactation is an essential aspect of motherhood, especially in the immediate post-partum. Multiple methods have been restricted in lactating mothers in the immediate post-partum period, hindering effective contraception; however, PPIUD insertion has no contraindications to its use during lactation and can be used freely in lactating mothers.[20]

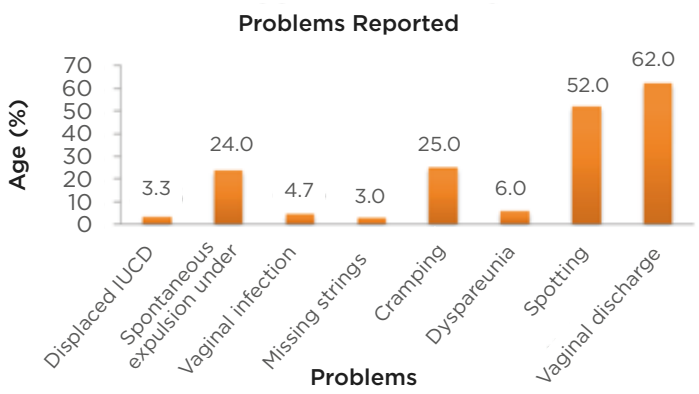
To overcome the risks to infant health and maternal health outcomes, in 2005, the WHO recommended a waiting time period of 24 months after birth before attempting the next pregnancy. The acceptability of post-partum IUCD insertions can be enhanced by developing awareness in the target population of these as an effective, hassle-free method of long-term contraception.

It was observed that, for most females, the husband was the decision-maker in family planning decisions and was playing a vital role in the choice of contraceptive methods to be chosen by the couple. Despite providing proper counselling to the females during the antenatal period, most of them did not follow what they had decided before. Therefore, counselling sessions for couples are recommended for females seeking hospital deliveries in order to prevent miscommunication and avoid a change of decision for the couples. The contraceptive method selection is equally important, so decision-makers should be aligned in

Table 1: Total births during study period.

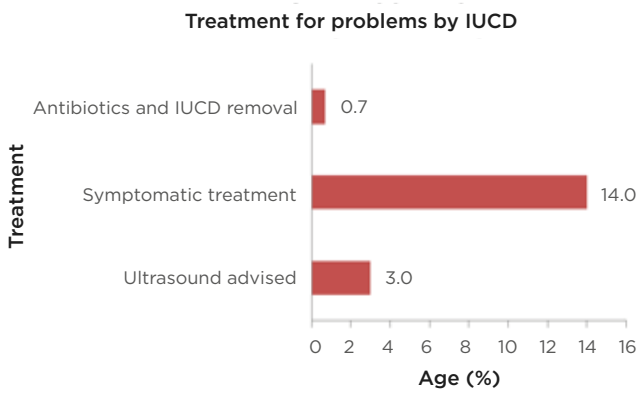
Total births	43,605
PPIUCD	5,275
Clinical follow-up	13%
Telephonic conversation	87%
Advised clinical visit	33%
No complaints	83%
Reassurance	11%
Thread trimmed	2%

Figure 1: Data of the issues reported.



IUCD: intrauterine contraceptive device.

Figure 2: Treatment for issues from the intrauterine contraceptive device.



IUCD: intrauterine contraceptive device.

their choice of method once they agree on contraception after childbirth.[21] In certain studies, it was noted that in some cases the mother-in-law opposed (1.4%), and in other cases, the husband did not allow for post-partum IUCD insertion (11.0%); some clients stated another reason (2.8%), such as a decline in sexual activity following the IUD insertion.

In Mexico, family planning has been included in antenatal services and counselling is substituted into routine pre-natal care. This change has brought improvement to the contraceptive practices, and the sterilisation rate is increasing significantly with pre-natal family planning counselling. Studies from Mexico prove this change is effective in the antenatal counselling of couples.[22] Although no randomised controlled trials have evaluated the expulsion rates of IUD insertion at the time of caesarean section delivery in comparison to vaginal delivery, it was found by one cohort study that there was a lower expulsion rate with IUD insertion at the time of delivery compared with insertion within 48 hours after birth.[23]

At a community level, misconceptions are still an issue. IUD has been associated with taboos and religious disbeliefs, but in multiple studies, satisfaction rates were extremely high, as well as continuation rates of post-placental IUD use.

CONCLUSION

PPIUCD has higher rates of expulsion and other minor complications compared with interval IUCD. It is a trade-off between greater contraceptive coverage versus a slightly higher complication rate; however, complications can be reduced by improving patient selection, comprehensive clinical follow-up, and early counselling. The use of PPIUCD offers an excellent window of opportunity for providing effective long-term contraception to the patients who need it most and the nations that are expected to have a population explosion.

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