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EMJ Reproductive Health

Review of ESHRE 2023

Editor's Pick

Vertical Transmission in Pregnancy with COVID-19 by Wiradnyana et al.

Interviews

Exclusive interviews with Stephen Franks, Diana Kuh, and Søren Ziebe

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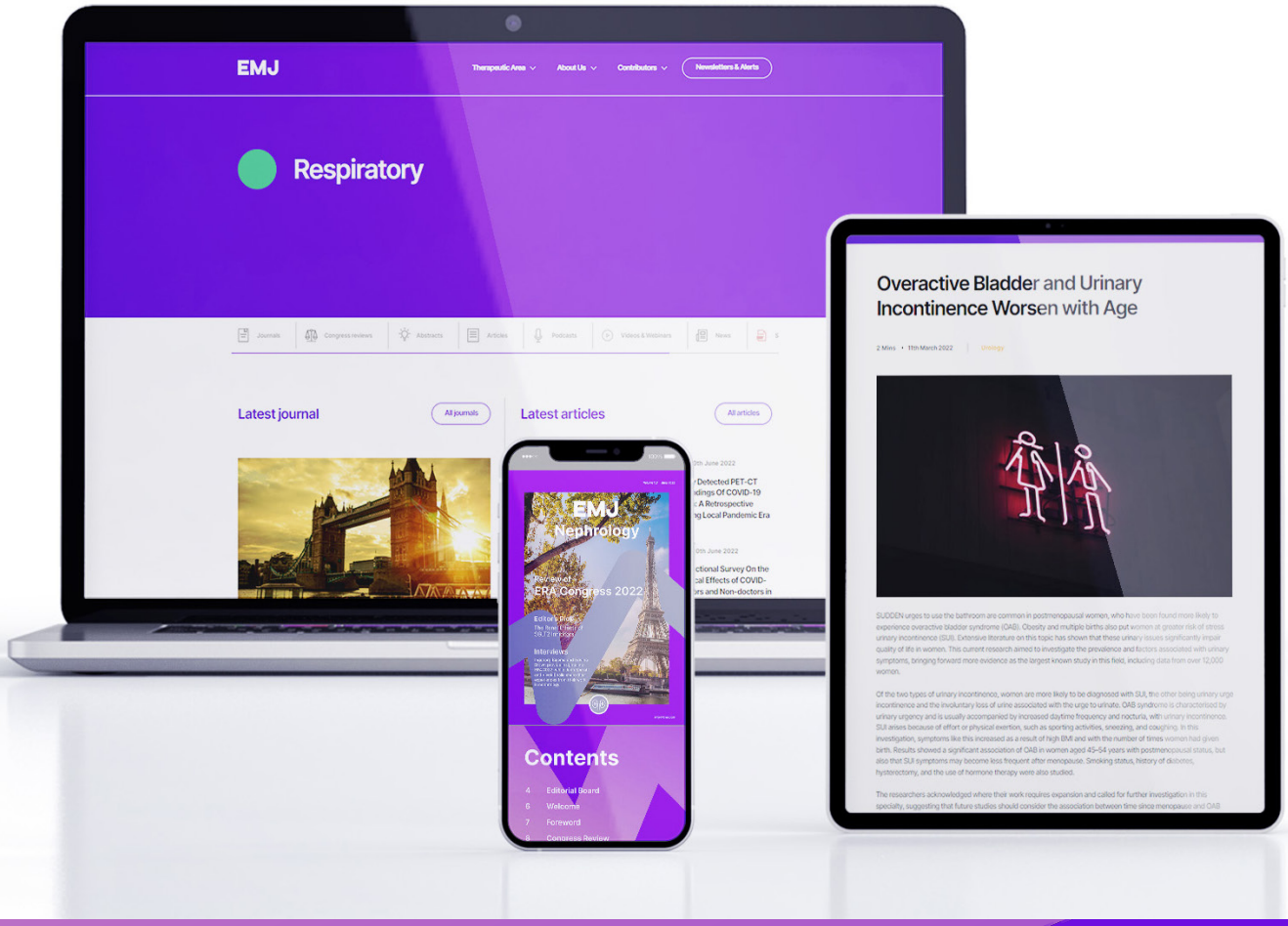

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Welcome letter

Koutsouki

Evgenia Koutsouki
Editor

Welcome to this issue of *EMJ Reproductive Health*. I would like to start by giving a warm welcome to our new Editor-in-Chief, Justin Chu, from the University of Birmingham, UK. Justin is an acclaimed expert in reproductive medicine and surgery, and is particularly interested in studying male fertility, embryo implantation in assisted reproductive treatments, miscarriage, and reproductive surgery. We look forward to a very fruitful collaboration!

In this issue, we are delighted to bring you the latest updates from the 39th European Society of Human Reproduction and Embryology (ESHRE) Annual Meeting, which took place in Copenhagen, Denmark. The topics that dominated the discussion are embryology, reproductive endocrinology, and andrology.

We are very proud of publishing our infographic on the relatively neglected topic of the menopause, providing information on the symptoms and stigma around this event in females' lives. The experts who were interviewed for this issue discuss developments in polycystic ovary syndrome, life course epidemiology, and assisted reproductive technology, and there is plenty to be learnt from the experiences that they share here. Among the articles included in this issue, you will find a feature that discusses the effects of exogenous endocrine disrupting chemicals on fertility, a very current and relevant topic.

I would like to close by thanking our fantastic Editorial Board, our contributors, and, of course, the peer reviewers who helped us to publish content of high quality. The EMJ team is excited for the year ahead working with our Editor-in-Chief, Justin Chu, to deliver more amazing content!

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Foreword

Dear Colleagues,

Welcome to our latest issue of *EMJ Reproductive Health*. I am very excited to be joining the EMJ team. This issue comprises a range of peer-reviewed articles, interviews with field experts, and features discussing unexplained fertility and stimulation in assisted reproductive technology. Also included is a review of the European Society of Human Reproduction and Embryology (ESHRE) 39th Annual Meeting, held in Copenhagen, Denmark between 25th–28th June. The review offers a detailed overview of the most significant content presented throughout the congress and the most exciting research abstracts.

EMJ had the pleasure of speaking to various field experts for this issue, namely Stephen Franks, who discussed his publications within the field of reproductive endocrinology and more specifically his work focusing on polycystic ovary syndrome. Søren Ziebe discussed the advances in assisted reproductive medicine, and finally, Diana Kuh discussed her career focusing on life course epidemiology and recent publications around female health and menopause.

The articles in this issue cover a range of topics. Begum et al. present a case report of ovarian hyperstimulation syndrome following the dilation and evacuation of a hydatidiform mole. Pranamartha et al. look at vertical transmission in pregnancy with COVID-19, while Tahir et al. present an unusual case of ovotesticular disorders of sexual differentiation with male and female external genitalia. Finally, D'Angelo and St Pier's feature presents the effects of endocrine disrupting chemicals on outcomes of pregnancy and fertility treatments, suggesting that this should not be underestimated.

Further content includes an infographic exploring the menopause. Specifically focusing on commonly reported symptoms, the causes and consequences of menopause are presented. Important statistics regarding female experiences with healthcare professionals are also reported with the aim of breaking the stigma.

As the new Editor-in-Chief, I thank all the authors, reviewers, and Editorial Board members for their contributions to this fantastic issue of *EMJ Reproductive Health*. I hope you enjoy reading this journal.

Justin Chu

Consultant Obstetrician and Gynaecologist, Sub-specialist in Reproductive Medicine and Surgery, Birmingham Women's and Children's NHS Foundation Trust, UK; Honorary Senior Lecturer, Institute of Metabolism and Systems Research, University of Birmingham, UK

ESHRE 2023

Review of the European Society of Human Reproduction and Embryology (ESHRE) Annual Meeting 2023

Location:	Copenhagen, Denmark
Date:	25th–28th June 2023
Citation:	EMJ Repro Health. 2023;9[1]:10-22. DOI/10.33590/emjreprohealth/10302219. https://doi.org/10.33590/emjreprohealth/10302219.

The 39th European Society of Human Reproduction and Embryology (ESHRE) Annual Meeting, which took place in Copenhagen, Denmark, welcomed 10,831 participants from 129 countries around the globe, with 90% attending in person. With a record number of 2,109 abstracts submitted, the main topics covered embryology, reproductive endocrinology, andrology, and more, which were certain to leave participants cleverer and more inspired.

With the decline in fertility rates worldwide and the needs for fertility treatments continuously increasing, the role of the ESHRE and its congress in emphasising high quality treatments, with a focus on efficient and safe treatments, as well as prevention, has become more important in recent years. At the opening ceremony, Anja Pinborg, Copenhagen University Hospital, Denmark, emphasised the proactive approach taken by Denmark, where this year's congress was hosted, by introducing fertility counselling into regions, and incorporating the importance of fertility awareness into the school curriculum. With these steps, they hope to spread the message of optimal timing of parenthood and its significance for both males and females.

ESHRE Chair, Carlos Calhaz-Jorge, shared the many activities of the Society, which aim to share knowledge and provide education, set standards, support research, and collect data. One example of this is the European Monitoring of Medical Assisted Reproduction (EuMAR) project, co-founded by the European Union (EU), which aims to develop a pan-European registry of data on the use of medical assisted reproduction treatments. This ambitious project has three objectives: to establish a data-flow that is flexible and adjusted to the context of all member states; to define, standardise, and harmonise parameters that will be collected, as well as the definitions; and to create new solutions establishing web-based data registry. Other activities of ESHRE that contribute to their goals include a portfolio that collects guidelines and good practice recommendations; the four journals published by the Society; the strong certification and standard setting programme; their support in research; and the strong policy-

"The main topics covered embryology, reproductive endocrinology, andrology, and more."

making awareness and advocacy programme, mainly with EU organisations, with new regulations coming out, and the need to be fully involved as stakeholders in the field.

This year, ESHRE granted six awards for content, which were presented at the meeting. The Basic Science Award for oral presentation was presented to Inmaculada Pérez-Prieto, University of Granada, Spain, for their work entitled 'Gut microbiome in endometriosis: a cohort study on 1000 individuals'. Second, the Clinical Science Award for oral presentation was awarded to Andrew Horne, Medical Research Council (MRC) Centre for Reproductive Health, Queen's Medical Research Institute, Edinburgh, UK, for their presentation entitled 'Combination of gefitinib and methotrexate to treat tubal ectopic pregnancy (GEM3): a multicentre, randomised, double-blind, placebo-controlled trial'. The winner of the Basic Science Award for poster presentation was Ying-Chun Guo, Reproductive Medicine Research Center, Yat-sen University, Guangzhou, China, for their presentation on 'Neurotrophin-4 supplementation during human secondary follicle in-vitro-culture supports morphologically normal blastocyst formation'. The Clinical Science Award for poster presentation was presented to Katja Drechsel, Cancer Center Amsterdam, the Netherlands, whose work was entitled 'Gonadal function and fertility preservation in girls with Hodgkin lymphoma treated according to the EURONET-PHL-C2 Protocol: the fertility add-on study'. ESHRE also presented the Fertility Society of Australia and New Zealand educational grant, allowing one of the participants to present their data at the Annual Meeting of the Fertility Society of Australia and New Zealand. This was awarded to Nada Kubikova, Nuffield Department of Women's and Reproductive Health, and Jesus College, University of Oxford, UK, for their work entitled: 'Deficiency of DNA double-strand break repair in human preimplantation embryos revealed by CRISPR-Cas9'. Finally, the Nurses Award was presented to Sarah Bailey, University Hospitals Southampton NHS Foundation Trust, UK, for their presentation entitled 'The Positive Reappraisal Coping Intervention: how it works in recurrent pregnancy loss'.

"Other activities of ESHRE that contribute to their goals include a portfolio that collects guidelines."

Read on for scientific highlights of the congress, covering topics such as the optimal embryo transfer time, and whether COVID-19 infection affects male fertility. EMJ was delighted to attend this congress, and is looking forward to the ESHRE's 40th Annual Meeting, which will be held next year from 7th–10th July in Amsterdam, the Netherlands. ●

Vaginal Progesterone Could Reduce Unexplained Fertility

UNEXPLAINED infertility affects approximately one-third of couples attending fertility services. These couples could benefit from a hormone treatment, suggests breaking research presented at the 39th ESHRE Annual Meeting.

The study, conducted at Queen Mary University of London, UK, included 143 couples with unexplained infertility. Pregnancy outcomes were compared between couples trying to conceive naturally and couples where females used a vaginal progesterone treatment during the second half of their menstrual cycle. All participants used ovulation test kits to plan intercourse for three menstrual cycles, with half of the females receiving 400 mg of progesterone twice daily via a vaginal suppository for 14 days.

Among the females treated with progesterone, 11 babies were conceived (15.3%), while five were conceived in the non-treated group (7%). The rate of miscarriage was 20% in the progesterone treatment group compared with 40% in the untreated group. Furthermore, there were no miscarriages among females who took progesterone according to the treatment protocol (throughout the second half of their menstrual cycle and early pregnancy with no breaks). However, the included population size is too small for researchers to claim that these results are statistically significant.

> **"Among the females treated with progesterone, 11 babies were conceived (15.3%)."**

Claudia Raperport, Queen Mary University of London, commented that "we need to do further research to prove these results in a larger group of people, but this trial suggests a potential treatment for couples with unexplained fertility. Given its safety and low price, there is no harm in offering this treatment in the meantime." Vaginal progesterone has been safely used for over 30 years and the cost of this progesterone treatment would be less than 200 EUR. Raperport therefore concluded: "The cost of progesterone is minimal compared to the cost of *in vitro* fertilisation and other fertility treatments. It also carries far less clinical risk, and physical and emotional burden for the couples involved." ●

What Is the Optimal Embryo Transfer Time?

NO DIFFERENCE in cumulative live birth rates is seen when cultured embryos are transferred after 3 or 5 days, but the results may be impacted by age, according to research presented at the ESHRE Annual Meeting, on 26th June 2023.

Transfer typically occurs after 5 days, once the embryos reach blastocyst-stage, as opposed to cleavage-stage (3 days); however, there are risks associated with increased culture time outside of the womb prior to transfer.

To evaluate if embryo culture time impacts live birth rate, researchers performed a large randomised controlled trial, which recruited 1,202 patients with ≥4 embryos available to be transferred after 2 days of culture, across 21 Dutch fertility centres, who were randomised to receive embryo transfer after either 3 days (n=599) or 5 days (n=603) of culture. Birth rate was derived from the transfer of either fresh or frozen embryos after one round of egg retrieval. The team also assessed cumulative live birth rate in those <36 years of age and those ≥36 years of age.

Overall cumulative live birth rates were 58.9% for blastocyst-stage and 58.4% for cleavage-stage embryo transfer, and there was no difference in time to achieve a pregnancy that resulted in live birth between the two groups.

When evaluating the impact of age, for those <36 years, the cumulative live birth rate for cleavage-stage embryos was 67% compared with 63% for blastocyst-stage embryos. However, this was not a statistically significant difference. In those ≥36 years of age, cumulative live birth rates for cleavage- and blastocyst-stage embryos was 43% and 52%, respectively. This difference was not found to be statistically significant. Whilst no statistical significance was reached, the authors commented that the results are still clinically significant and highlight a potential benefit of cleavage-stage transfer in females aged up to 36 years, as well as a potential benefit of blastocyst-stage transfer in females ≥36 years.

"Cumulative live birth rates were 58.9% for blastocyst-stage and 58.4% for cleavage-stage embryo transfer."

Live birth rates following fresh embryo transfer alone were significantly higher in the blastocyst-stage than the cleavage-stage at 37.0% and 29.5%, respectively. This difference was more apparent when stratifying by age. Live birth rate in patients ≥36 years was 35.0% for blastocyst-stage and 18.5% for cleavage stage. However, in patients <36 years of age the difference was not significant, at 38.0% and 36.0% for blastocyst- and cleavage-stage embryo transfer, respectively.

The team emphasised the importance of evaluating new techniques in clinical trials before introducing into clinical practice and looking towards the future. They are conducting a cost-benefit analysis and plan to assess the risks and burden of treatment to patients.

Cancer Risk Greater in Females with Polycystic Ovary Syndrome After Menopause

FEMALES with polycystic ovary syndrome (PCOS) who have been through menopause are more than twice as likely to be diagnosed with ovarian cancer compared with those without this condition, according to data presented at the 39th ESHRE Annual Meeting.

Data presented was based on close to 2 million females, the first large-scale study of its kind, aiming to increase awareness for the management of health in females with PCOS, which affects one in 10 females. This study was led by Clarissa Frandsen, Danish Cancer Research Center, Copenhagen, Denmark, who advocated for clinical guidelines including recommendations on the potential ovarian cancer risk when managing the long-term health consequences of patients with PCOS. They stated: "Our results and those from previous studies should be taken into account when revising guidelines on how to manage the health of women with PCOS," going on to stress that, "unfortunately, there is no effective screening for early detection of ovarian cancer. Both patients and clinicians will benefit from improved knowledge of the potential long-term health risks associated with PCOS."

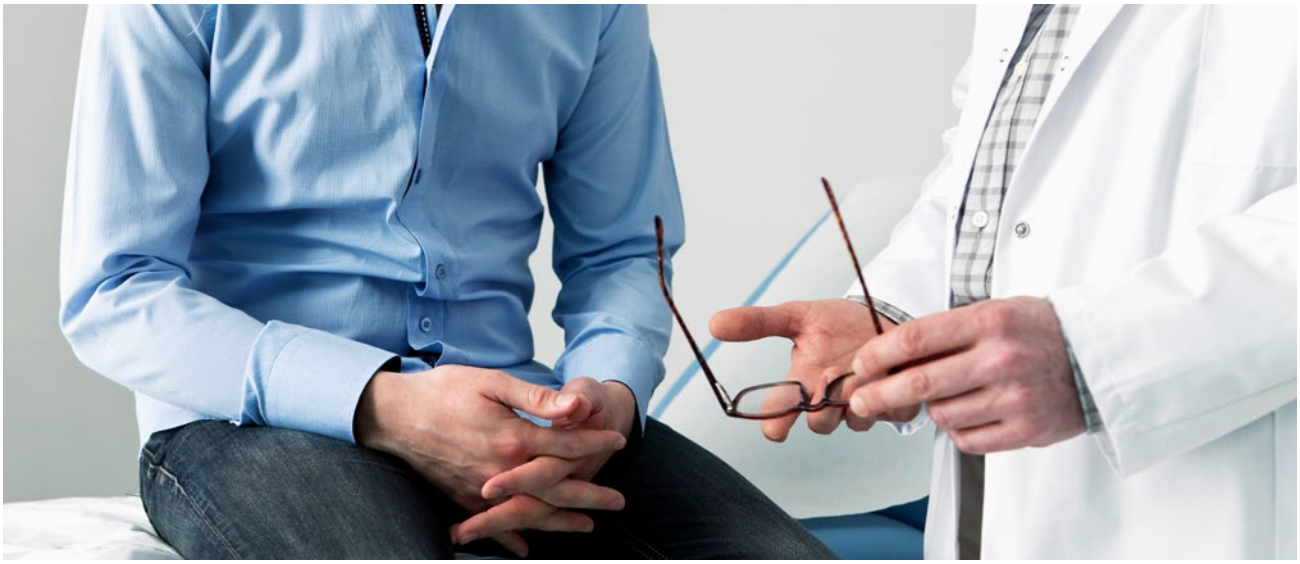
Age-adjusted incidence rates of ovarian cancer were 11.7 per 100,000 person-years and 13.2 per 100,000 person-years for females with and without PCOS, respectively. The cohort under surveillance included 1.7 million females born in Denmark between 1st January 1940–31st December 1993. Excluded from this population were females who emigrated, died, were diagnosed with cancer, or underwent surgery to remove their ovaries/fallopian tubes. Details about diagnosis of PCOS and cancer, as well as migration, were obtained from national registers.

Further analysis was performed on females reaching 51 years, which is the average age in Denmark for menopause.

> **"The risk of developing ovarian cancer was significantly greater among post-menopausal females."**

In total, 6,490 females were diagnosed with epithelial ovarian cancer, and 2,990 with borderline ovarian tumours, over a median follow-up time of 26 years. The investigators discovered that increased risk was not statistically significant for ovarian cancer and borderline ovarian tumours among females with PCOS compared with those without the condition. Other factors, such as obesity and education level, were considered as potentially affecting the risk of ovarian cancer. Notably, the risk of developing ovarian cancer was significantly greater among post-menopausal females compared with those without PCOS. Plus, risk of a serious borderline ovarian tumour was more than doubled among patients with PCOS.

The authors of this work did acknowledge the low number of ovarian cancer cases, despite a large study population. This research did not examine why post-menopausal females are more likely to develop ovarian cancer, and future study is warranted to delve deeper into this area. Frandsen described PCOS as a complex condition and suggested that long-term exposure to potential cancer-causing factors could be behind findings, such as excess production of male sex hormones. ●



Does COVID-19 Infection Impact Male Fertility?

SEMEN quality is reduced long-term following COVID-19 infection, according to new research presented at the 39th ESHRE Annual Meeting on 26th June 2023.

The study, led by Rocio Núñez-Calonge, UR International Group, Scientific Reproduction Unit, Madrid, Spain, enrolled 45 males with a confirmed diagnosis of mild COVID-19 who had available data on semen analysis pre-COVID-19 infection, from six reproductive clinics in Spain between February 2020–October 2022. A second semen sample was taken between 17–516 days post-infection. Median patient age was 31 years and median duration between pre- and post-COVID-19 infection semen samples was 238 days.

Semen samples taken within 100 days post-infection were analysed separately to those taken >100 days. The results revealed that following COVID-19 infection there was a statistically significant reduction in semen volume, total motility, sperm concentration, sperm count, and number of live sperm, with sperm count and total motility being most affected, according to Núñez-Calonge.

Analysis of patients who had a semen sample within 100 days of infection found that on average, sperm count reduced by 37.5%, sperm concentration by 26.5%, semen volume by 20%, total motility by 9.1%, and live sperm numbers by 5.0%. However, the shape of sperm was not found to be significantly affected. Half of those in the study experienced a 57% reduction in total sperm count in their post-infection sample compared to their pre-infection sample.

> **"Half of those in the study experienced a 57% reduction in total sperm count in their post-infection sample."**

Separate analysis of patients who had a post-infection semen sample taken >100 days after infection showed that sperm concentration and motility had still not improved at this time. Núñez-Calonge highlighted that this prolonged impact on semen quality could be secondary to permanent damage caused by COVID-19 infection. However, Núñez-Calonge noted that impairment of semen quality may not be a direct effect of severe acute respiratory syndrome coronavirus 2, and studies have previously shown that COVID-19 infection can impact testosterone levels, which were not measured during the study.

This study highlights how long-term follow-up following COVID-19 is important in understanding the impact of infection on male fertility. The researchers plan to continue their research by evaluating semen quality and hormone levels temporally. ●

Risks of Gene Editing in Early Human Embryos

CAUTION should be adopted when considering gene editing techniques to remove inherited diseases from human embryos, according to new research presented at the 39th ESHRE Annual Meeting.

To determine if the gene editing method, CRISPR-Cas9, is safe to use for human embryo genetic error correction, Nada Kubikova, University of Oxford, UK, and fellow researchers created 84 embryos using intracytoplasmic sperm injection to fertilise donor eggs with donor sperm. CRISPR-Cas9 was used to create double-strand DNA breaks in areas containing no genes in 33 of the embryos. The remaining 51 were kept as controls.

The desired method of DNA repair to limit errors and mutations is homologous directed repair. Non-homologous end-joining has a greater risk of introducing mutations than homologous directed repair. However, complete failure to repair DNA damage can be lethal. In their study, the researchers found that alterations at the targeted DNA sites occurred in 24/25 embryos, highlighting the efficacy of CRISPR. Repair at the targeted DNA sites occurred via non-homologous end-joining in 51% and by homologous directed repair in 9%. The remaining 40% displayed no repair, which resulted in large chromosomal areas being deleted or duplicated. This can impact embryo viability and lead to serious congenital abnormalities.

These findings highlight that repair of DNA damage by homologous directed repair is limited in early human embryos and that DNA repair during these early stages is not optimal. The majority of repair that did occur in the study, took place by non-homologous end-joining, resulting in introduction of additional mutations. This suggests that in the majority of human embryos with inherited disorders, CRISPR-Cas9 is unlikely to be a successful gene editing technique.

"Repair of DNA damage by homologous directed repair is limited in early human embryos."

Kubikova stated: "Our new findings provide a warning that commonly-used gene editing technologies may have unwanted and potentially dangerous consequences if they are applied to human embryos." The study findings may aid understanding of *in vitro* fertilisation embryo failure and subsequently lead to improved *in vitro* fertilisation treatments.

Looking towards the future, the researchers plan to identify methods of protecting early human embryos against DNA damage and explore other gene editing techniques that avoid DNA strand breakage.

Why Are So Many Frozen Eggs Not Used?

RESEARCH presented at the 39th ESHRE Annual Meeting has demonstrated that whilst over 40% of females who chose to freeze their eggs during their 30s were able to have children later in life, many females did not return to the fertility clinic, and others chose fertility treatments that did not involve using their own frozen eggs.

The study, which focused on females who underwent elective oocyte cryopreservation, was carried out by Ezgi Darici and colleagues from the Centre for Reproductive Medicine, Universitair Ziekenhuis (UZ) Brussels, Belgium. In total, 843 females who had elective oocyte cryopreservation for reasons that were not medical at the Centre for Reproductive Medicine between 2009–2019 were included. Mean age was 36 years and the majority of females were single.

As of May 2022, 231 females (27%) in the cohort had returned to the same centre for treatment. Mean age upon their return to the clinic was 40 years, and the majority had partners. Of these, 110 (48%) used their own frozen eggs in their fertility treatment, 50 (22%) underwent intrauterine insemination, and 71 (31%) had fertility treatments like *in vitro* fertilisation, using fresh eggs. In this subgroup, 106 females (46%) had a live birth (a cumulative live birth rate included every live birth following any fertility treatment), and the rate of miscarriage was 31%.

Of those using frozen eggs in their treatment, 41% had live births, and with fresh eggs, this rose to 48%.

"Of those using frozen eggs in their treatment, 41% had live births, and with fresh eggs, this rose to 48%."

Darici commented: "To our knowledge, this is one of the first and largest reports of reproductive outcomes in women who had elective oocyte cryopreservation at a European fertility centre." Darici went on: "The choice of whether to use fresh or frozen eggs is made based on what treatment is best for each individual woman, and factors such as the woman's age are important. We cannot really compare the two groups, as there will be many differences that could underlie any disparity in pregnancy and birth rates."

The study found positive rates of pregnancy and birth in fertility treatment using both fresh and frozen eggs. Limitations of the study are the small sample size and the use of retrospective data. Carlos Calhaz-Jorge, Northern Lisbon Hospital Centre and Hospital de Santa Maria, Lisbon, Portugal, and Chair of ESHRE, stressed that more research was needed to prove that freezing eggs successfully aided fertility in older females.

Childhood Hodgkin Lymphoma Treatment May Cause Early Fertility Decline

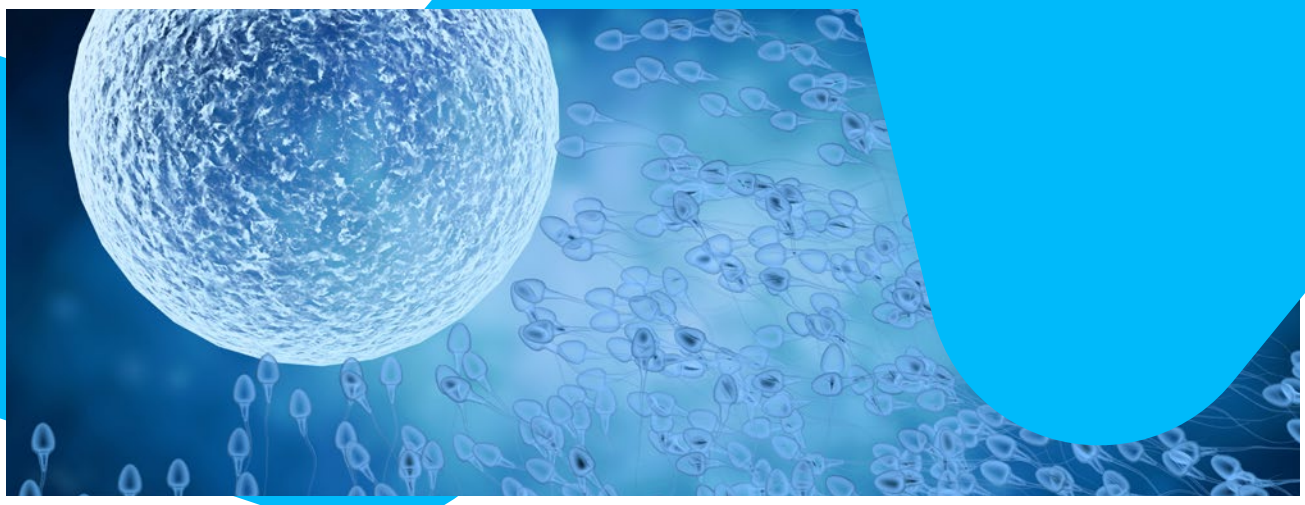
FERTILITY of patients who were treated for Hodgkin lymphoma in childhood may decline at a younger age, according to a study presented by Katja Drechsel, Princess Máxima Center for Paediatric Oncology, Utrecht, the Netherlands, at the ESHRE Annual Meeting 2023. However, data also showed that the majority of those who tried to get pregnant were ultimately successful. Most people with Hodgkin lymphoma survive thanks to improvements in treatment; however, treatments such as radio- and chemotherapy can reduce fertility.

In total, 84 females who were treated for Hodgkin lymphoma as children, and 798 who were not treated for this condition were included in the study. Participants were asked whether they had children and at what age they first became pregnant. They were also tested for markers of fertility, such as anti-Müllerian hormone, follicle-stimulating hormone, and inhibin, and the number of egg cells in the ovaries were gauged by ultrasound. Data showed that those who were treated for the condition were more likely to have abnormal fertility markers and a lower number of egg cells compared with those who were not. Furthermore, those who survived Hodgkin lymphoma were almost 2.5-times more likely to try for 1 year before becoming pregnant for the first time.

Researchers noted similar pregnancy and live birth rates in both groups, but females who were treated for Hodgkin lymphoma were on average 2 years younger when they had their first child compared with the control group. Researchers hypothesise that this may be due to doctors advising them about the effects of cancer treatments on fertility.

The team concluded that treatment for childhood Hodgkin lymphoma may lead to an earlier decline of fertility. Drechsel noted: “These women were treated for cancer in the 1970s, 80s, and 90s. It is important to note that treatment for childhood Hodgkin lymphoma has changed in recent years and the effects of current treatment schedules on fertility are likely to be less toxic.” More research is needed to follow patients’ fertility long-term and determine if they have more difficulties becoming pregnant at an older age. ●

"Those who survived Hodgkin lymphoma were almost 2.5-times more likely to try for 1 year before becoming pregnant."



Artificial Intelligence Accurately Detects Sperm in Males with Infertility

NEW research presented at the 39th ESHRE Annual Meeting heralds a new artificial intelligence (AI) tool which has the ability to identify sperm in males with the most severe form of infertility, non-obstructive azoospermia, where no sperm is detectable in semen.

In current practice, patients have to undergo a procedure to remove a portion of their testes. Tissue is partially shredded, and sperm is extracted manually by an embryologist, which is then used to fertilise their partner's eggs through intracytoplasmic sperm injection. Currently, it can take as long as 6 hours to detect and isolate sperm in human tissue; this makes it more difficult to identify, due to mental and physical fatigue on the part of the embryologist. Contamination from other tissue particles can also make this process difficult. The longer this process takes, the higher the chance that the sperm will not be viable for treatment.

> **"The algorithm improves antiquated approaches that have not been updated in decades."**

Research was carried out at an *in vitro* fertilisation clinic in Sydney, Australia, over 5 months, using a two-stage process. Researchers initially trained the AI algorithm using thousands of still microscope photographs, featuring sperm and high levels of other cells; only the sperm was highlighted in this process. The AI tool was able to learn, through image analysis, what sperm looked like using an evaluation system able to check and adjust its performance. Healthy sperm, along with testicular tissue from seven patients aged 36–55 years, all of whom had been diagnosed with non-obstructive azoospermia, were used.

In several seconds, the SpermSearch (University of Technology Sydney, Australia) AI tool can instantly identify sperm. An embryologist can then decide whether intracytoplasmic sperm injection treatment is viable. AI detected more sperm overall, but some were found only by the embryologist (560), and some by AI alone (611). AI found 60 more sperm overall, and was 5% more accurate than the embryologist when based on viewable droplet area.

Lead author Dale Goss, University of Technology Sydney, commented: "The algorithm improves antiquated approaches that have not been updated in decades. It will ensure the rapid identification of sperm in samples, which will not only increase the chance of a couple conceiving their own biological children, but also reduce stress on sperm, and increase efficiency in the laboratory."

It is hoped that this algorithm will bring hope to males with severe infertility who wish to have a biological child, but have no sperm detectable in their semen.

Data Suggests More Patients Are Using Single Embryo Transfer

PRELIMINARY data shared at the 39th ESHRE Annual Meeting shows that more females are having just one embryo transferred per cycle of fertility treatment in order to get pregnant. In 2020, nearly three out of five (57.6%) of all *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection procedures in Europe involved the transfer of just a single embryo. This number represents an increase over the half (55.4%) who used single embryos in 2019.

Corresponding to the reduction in multiple embryos, there was a resultant increase in singleton babies, which accounted for 88.8% of all assisted reproductive technique deliveries compared with 87.7% the previous year. There was a decreased number of twins (11.0%) and triplets (0.2%) from the previous year, 11.9% and 0.3%, respectively.

"Corresponding to the reduction in multiple embryos, there was a resultant increase in singleton babies."

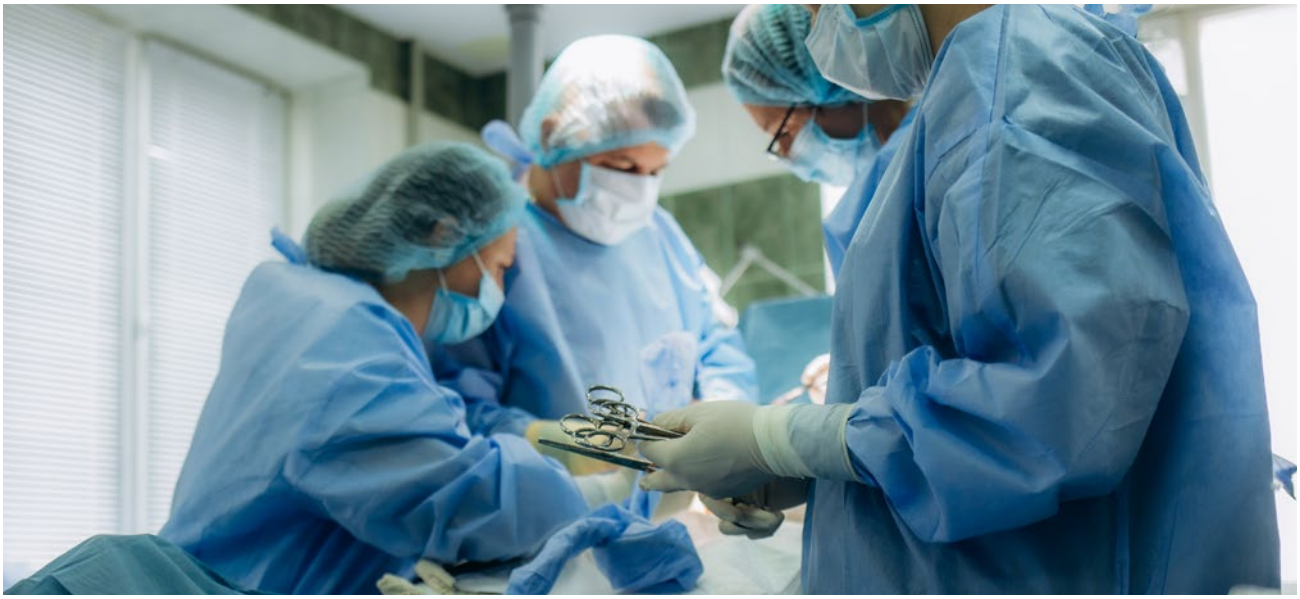
This ESHRE European IVF-monitoring Consortium (EIM) report represents the largest data collection on medical assisted reproductive techniques in Europe, with lead author Jesper Smeenk, Elisabeth-TweeSteden Ziekenhuis (ETZ), Tilburg, the Netherlands, stating: "The continued rise in single embryo transfer means women are less likely to face complications in pregnancy and during birth. The result has been that fertility treatments have become safer for mothers and babies without compromising success rates."

Additionally, according to research from 1,326 clinics in 38 European countries, the number of treatment cycles throughout 2022 dropped compared to the year before. A total of 843,776 cycles occurred in 2020 compared with over 1 million in 2019. However, the presenters stressed that the number of cycles is likely to increase once the full data is reported.

The report also provided data on the frequency of fertility preservation methods. In total, 15 countries carried out a total of 18,270 fertility preservation procedures, including egg, sperm, and ovarian tissue freezing. These techniques, often used to allow for pregnancy in patients with cancer, were carried out both pre- and post-puberty.

The authors highlighted that their findings were not yet complete due to incomplete data returns from several countries, including the UK. They therefore encouraged interpretation of the results with a degree of caution.

"The hope is that this upwards trend in single pregnancies, as highlighted by the EIM data, continues," stated Carlos Calhaz-Jorge, Northern Lisbon Hospital Centre, Portugal. "Clinics must always prioritise the safety of patients who undergo fertility treatment, and that of their offspring."



Adenomyosis Linked to Increased Risk During Pregnancy

FEMALES with adenomyosis have an increased risk of problems during pregnancy and birth, according to research presented at the 39th ESHRE Annual Meeting. Lead author, Mohammed Bazarah, University of Western Ontario, London, Canada, stated that females with adenomyosis are more likely to "experience infertility, pre-term delivery, and other gynaecological conditions, such as endometriosis."

A chronic condition similar to endometriosis, adenomyosis causes heavy menstrual bleeding and pelvic pain. Occurring when endometrial tissue and glands that line the womb grow into or are found in the uterus' muscle wall, one in 10 females of reproductive age have adenomyosis. However, some females have no physical signs, making the condition difficult to diagnose.

"One in 10 females of reproductive age have adenomyosis. However, some females have no physical signs."

Using records from the Nationwide Inpatient Sample (NIS) database from 2004–2014, the researchers analysed 2,467 pregnant females with adenomyosis and 9,094,321 females without the condition. The outcomes of both cohorts were then compared. Using this data, the researchers wanted to provide insights into maternal, pregnancy, and neonatal outcomes in females with the condition.

The results indicate that females with adenomyosis were 1.69-times and 1.5-times more likely to develop pre-eclampsia and hypertension, respectively. Females with adenomyosis have a 5.86-times higher risk of placenta previa, with a relative risk of obstetric complications for Caesarean section being 21.63-times higher than females without adenomyosis.

Furthermore, the results showed that females with adenomyosis were more likely to have had a previous Caesarean section and *in vitro* fertilisation treatment, as well as have pre-gestational diabetes, chronic hypertension, and thyroid disease. Relative risk was also higher for hysterectomy, wound complications, blood transfusions, placenta detachment, excessive bleeding after the birth, and maternal infection in females with adenomyosis.

While the database used did not provide disease severity or how adenomyosis was diagnosed, the researchers believe that more females with adenomyosis need to be monitored worldwide to help reduce the risk of serious complications, such as death of the mother and their baby.

Guidelines on Unexplained Infertility

Authors: Evgenia Koutsouki, EMJ, London, UK

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DURING the 39th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), a new set of guidelines for couples with unexplained infertility (UI) was presented by Daniela Romualdi, Agostino Gemelli Polyclinic Foundation, Rome, Italy, and Siladitya Bhattacharya, University of Aberdeen, UK.

METHODOLOGY AND DEFINITION

Romualdi began by stating that putting the guidelines together included gathering evidence of efficacy and evidence of safety, with a literature search up to October 2022; and then further assessing the quality of papers, and formulating the recommendation based on the opinion of the experts, as well as the patient perspective.

Romualdi explained that UI is defined by ESHRE as infertility in couples with adequate coital frequency (at least 12 months of regular unprotected intercourse), where the female partner is aged 40 years or below, and has an apparently normal ovarian function, fallopian tubes, uterus cervix, and pelvis; and the male partner has apparently normal testicular function, genitourinary anatomy, and ejaculate. Inaccuracy of the diagnosis of UI rises sharply at 40 years of age, and there is an 80% chance of false diagnosis of UI at this age, meaning females could be subjected to inappropriate treatments.

RECOMMENDATIONS

The Guideline Development Group (GDG) recommends routinely taking a thorough medical reproductive and sexual history from both partners in couples with UI. This should be followed by confirming ovulation, which is not recommended in females with regular menstrual cycles.

If, however, in this group of females, confirmation of ovulation is warranted, the recommended tests are urinary luteinising hormone measurement, ultrasound monitoring, or mid-luteal progesterone measurement. Given the lack of biomarker for oocyte and corpus luteum quality, routinely measuring mid-luteal serum progesterone levels is not recommended. There is a strong recommendation against endometrial biopsy for histological examination in the absence of other indications.

In females with regular menstrual cycles, ovarian reserve testing is not required to identify the aetiology of infertility, or to predict the probability of spontaneous conceptions over 6–12 months. A strong recommendation is, however, using hysteron contrast sonography, hystero-foam sonography, or hysterosalpingography for testing tubal patency compared to laparoscopy and chromopertubation.

Chlamydia antibody testing could be considered for tubal patency, as it is a non-invasive method to differentiate between patients at low- and high-risk for tubal pathology. Tubal flushing with oil-soluble contrast medium is preferable over water-soluble contrast medium, but the risks of the former should be discussed with couples. In patients at high risk for tubal abnormality, visual demonstration of tubal patency is necessary.

"Chlamydia antibody testing could be considered for tubal patency."

"The GDG advises that the decision of introducing active treatment should be based on the prognosis of couples."

Routine diagnostic laparoscopy is not recommended for the diagnosis of UI. Laparoscopy should be reserved for females with an abnormal hysterosalpingography, or those at risk of tubo-peritoneal disease due to history of pelvic inflammatory disease, previous ectopic pregnancy, or clinically suspected or known endometriosis. A 3D ultrasound is recommended for excluding uterine anomalies in females with UI. However, no additional diagnostic procedures should be performed to confirm an anatomically normal uterine cavity.

There is a strong recommendation against postcoital test, as this is subjective, poorly reproducible, inconvenient to the patients, and can even change clinical management, but does not change the ability to conceive in couples with UI. Vaginal microbiota testing could be considered in these couples only for research purposes.

The GDG recommends at least one basic semen examination, performed by a laboratory that subscribes to an external quality control programme. If this result is below the lower fifth percentile reference limit according to the World Health Organization (WHO) criteria, a second analysis should be performed after a 3-month interval. Additional diagnostic procedures to confirm normal genitourinary anatomy in males before being diagnosed with UI are not recommended. There is also no added value of additional tests in males with normal WHO semen analysis for anti-sperm antibodies, sperm DNA fragmentation, chromatin condensation, aneuploidy screening, hormonal testing, human papillomavirus testing, and microbiology testing.

Testing for anti-sperm antibodies in couples with UI is not recommended, whereas testing for coeliac disease in females with UI can be considered. Testing for thyroid antibodies and other autoimmune conditions in females is not recommended. Even though thyroid-stimulating hormone measurement is considered good practice in preconception care, no additional thyroid evaluation is recommended if thyroid-stimulating hormone is within the normal range. Finally, testing for thrombophilia, oxidative stress, vitamin D deficiency, prolactin, and performing genetic/genomic tests, are not recommended in females with UI. Measurement of oxidative stress in semen of males in UI should only be considered in the context of research, and BMI evaluation is considered good practice in pre-conception care.

Romualdi summarised the main points, emphasising that, on the female side, menstrual history is enough to confirm regular ovulation, 3D ultrasound is enough to confirm normal uterine cavity structure, and that patency of the tubes can be verified by hysterosalpingo contrast sonography, hysterosalpingography, and chlamydia antibody test. For the male, a normal semen analysis based on WHO criteria may be sufficient to make diagnosis of UI.

MANAGEMENT OF UNEXPLAINED INFERTILITY

Bhattacharya spoke about the guidelines, highlighting the lack of high-quality evidence on many aspects of UI management. In a couple with UI and good prognosis, there is a period of expectant management, during which the couple try to conceive on their own. In couples with poor prognosis, the next step is ovarian stimulation (OS), and after three–six cycles of this, *in vitro* fertilisation (IVF). This pathway is also undertaken in couples who follow expectant management for more than 6 months, where this does not result in pregnancy. Bhattacharya highlighted that the decision to move straight to IVF without OS first can be made under exceptional circumstances.

EXPECTANT MANAGEMENT

Bhattacharya emphasised the importance of the decision of when to introduce active treatment after expectant management. Intrauterine insemination (IUI) with OS is the first-line active treatment recommended for couples with UI. The GDG advises that the decision of introducing active treatment should be based on the prognosis of couples. Bhattacharya highlighted that UI is not sterility, which is something that the couples would need to be reminded of, and that the chance of conception changes over time and is not static, as shown by studies done in couples with UI.

Two things to be considered when transitioning from an expectant approach to an active approach are the chances of a live birth with expectant management, and the additional benefit treatment provides. The treatment benefit can be estimated by subtracting the chance of live birth through expectant management from that of treatment, both of which are based on prognostic factors for each couple. Dynamic models have recently been developed in order to recalculate the new chance of conception over the next 12 months for couples who have previously been given such an estimate.

EXPECTANT MANAGEMENT VERSUS ACTIVE TREATMENT

Clomiphene use in couples with UI shows no benefit in live birth outcomes, and IUI without OS versus expectant management does not conclusively show a benefit. The outcomes of IUI with ovarian stimulation versus expectant management seem to vary based on the initial prognosis of the couple. Studies have shown that the same treatment can be effective in a group with a certain prognosis, but not in another.

Treatment in naïve females given either IVF or stimulated IUI showed no difference in outcomes. In a group of females previously treated with IUI, IVF showed obvious superiority, demonstrating again that the same treatment has different effects in different prognostic groups of patients with UI. A more recent trial showed no difference between three cycles of IUI and OS versus one cycle of IVF. Cumulative incidence of pregnancy leading to live birth is doubly in favour of IVF versus OS with IUI. Finally, comparing IVF with expectant management is still inconclusive due to small sample sizes; however, the odds ratio is 2.56 in favour of IVF.

ALTERNATIVE THERAPEUTIC APPROACHES

Conditional guidance says that adjunct oral antioxidant therapy for females and males undergoing fertility treatment is probably not recommended for either. The same applies to acupuncture in females, and nutraceuticals such as inositol supplementation. Psychotherapy is recommended for patients when needed, as are diet, exercise, and behavioural therapy.

NEEDS FOR FUTURE RESEARCH

According to Bhattacharya, three main questions need to be answered by future research:

- Can a predictive model be developed, tested, and validated to compare the outcomes of different management strategies of couples with UI?
- What is the optimal assistive reproductive technology for UI?
- What is the value of performing current methods to assess sperm DNA integrity, and to predict clinical outcomes in couples with UI?

In their concluding remarks, Bhattacharya highlighted that couples with UI have a real chance of natural pregnancy, and for this reason, a prognosis-based approach to active treatment that works out the net benefit of a proposed treatment in each couple is needed. OS and IUI remain the first line of active management, whereas IVF (not intracytoplasmic sperm injection) remains the final port of call. Finally, Bhattacharya explained that an individualised approach should be adopted, which takes female age and duration into account.

Debate: Mild Versus Conventional Stimulation in Assisted Reproductive Technology

Authors: Abigail Craig, EMJ, London, UK

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An exciting session, delivered at the 39th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), saw a debate regarding mild and conventional stimulation in assisted reproductive technology (ART). Chaired by Barış Ata, Koç University, Istanbul, Türkiye, and Mette Tanvig, Odense University Hospital, Denmark, the accessibility, efficiency, cost, and complications associated with mild and conventional stimulation were discussed, with thought-provoking, and often conflicting, opinions considered.

MILD STIMULATION

Geeta Nargund, St George's Hospital, London, UK, kicked off the debate, suggesting mild stimulation is the future of ART. Nargund reminded the audience that the aim of an *in vitro* fertilisation (IVF) cycle is to achieve a singleton, healthy, full-term baby with minimal health risks, at a reasonable cost that is within financial reach of most females globally, not just in the affluent world. Ovarian stimulation significantly contributes to the cost of IVF, while also being associated with significant side effects, discomfort, and the overall treatment burden. Healthcare professionals therefore have an obligation to consider both their scientific responsibility and their social responsibility to make this treatment accessible globally, while minimising the treatment burden.

Mild stimulation denotes daily ovarian stimulation at less than 150 IU, with or without oral medication, in a gonadotropin-releasing hormone antagonist cycle. The dose is not guided rigidly by oocyte number or a fixed daily dose; high responders have their dose adjusted accordingly.

A systematic review and meta-analysis published in 2020 showed that mild IVF is as effective as conventional IVF, reporting no difference in live birth rates, whereas mild IVF significantly reduces the risk of ovarian hyperstimulation syndrome (OHSS).[1] This study also demonstrated that cumulative live birth rate is not significantly different when comparing the two methods of stimulation, based on five randomised control trials. Further analysis also suggested the proportion of high-grade embryos is no different between mild and conventional stimulation.

Nargund also emphasised that mild IVF is associated with a reduced cost for poor, normal, and high responders, and more specifically, the cost per live birth is lower. A healthcare economics analysis investigating three studies reported a reduced cost for mild IVF, an important result when considering the accessibility of IVF globally.

Recent studies have reported that a higher number of oocytes retrieved is associated with an increase in the number of fertilised oocytes and the cumulative live birth rate.[2,3] However,

"Ovarian stimulation significantly contributes to the cost of IVF, while also being associated with significant side effects."

putting the studies in context, Nargund explained that the first study suggests that moderate-to-severe OHSS increases with the number of oocytes retrieved, while providing no information on ovarian stimulation protocols, patient burden, or cost, despite this data often being available in the database.

Furthermore, only the first live birth associated with the retrieval is accounted for in the cumulative live birth rate calculation, which suggests the cumulative live birth rate was increased in younger patients with high anti-Müllerian hormone; this is an unsurprising response, according to Nargund. The second paper again suggests that moderate-to-severe OHSS increases with the number of oocytes retrieved, but again neglects to analyse cost and patient burden data. Furthermore, females with a higher cumulative live birth rate had significantly reduced gonadotrophin doses per day. In other words, females who fell within a threshold considered to be 'mild stimulation' had a higher cumulative live birth rate. The authors conclude that while very high ovarian response may further increase cumulative live birth rates, ovarian stimulation should be rational, and avoid extreme response, recommending against an extreme oocyte yield.

Nargund summarised by questioning the need for conventional stimulation altogether. They highlighted that only 22.4% of females achieve enough oocytes to expect more than one child, meaning three-quarters of females would be exposed to high stimulation and high cost unnecessarily. Furthermore, data suggest around 20% of females have a child naturally in the 3 years following an IVF treatment cycle. Thus, high stimulation to try and "fill freezers full of unwanted embryos" is unnecessary, and mild stimulation is favourable, as it gives an equivalent success rate whilst minimising risks such as OHSS.

CONVENTIONAL STIMULATION

Ernesto Bosch, Instituto Valenciano de Infertilidad, Valencia, Spain, next presented conventional stimulation, naming it the 'gold standard'. They began by outlining that the concept of conventional ovarian stimulation, which aims to optimise ovarian response, ensuring the best possible embryo cohort, maximises the live birth rate per cycle. Mild stimulation, developed 20 years ago, has three main cornerstones: increased oocyte-embryo quality, lower risk of OHSS, and lower cost.

"Mild stimulation has three main cornerstones: increased oocyte quality, lower risk of OHSS, and lower cost."

However, Bosch suggested that this is not relevant to current practice, as efficiency has improved greatly.

First, tackling the concept of increased embryo quality, Bosch considered a publication by Baart et al.[4] They concluded that surplus embryos generated were of low quality and not viable. In theory, fewer eggs (between three and six) would therefore optimise implantation rates. However, a paper published more recently, following the introduction of vitrification, analyses 14,469 patients. Results suggest that collecting more eggs increases the cumulative live birth rate in this case.[3] A further publication, looking at 402,411 cycles, also supports this, with both the cumulative live birth rate and pregnancy rate increasing with the number of oocytes retrieved.[5] Concurrently, as the number of oocytes retrieved increases, the number of embryos, blastocysts, and euploid blastocysts increases. Bosch therefore summarised that if you stimulate the ovary mildly, the best oocytes will not be collected. They commented that the ovary "is not going to make that selection," and the same oocytes will be produced using both methods of stimulation. "You change the magnitude of the cohort, you don't change the quality of the cohort," when comparing mild and conventional stimulation. This finding remains when stratifying by age, as demonstrated by Fanton et al.[2]

To further support the case of conventional stimulation, it is important to prove that the oocytes are not harmed by either method. Bosch presented a prospective cohort study of 40 infertile patients aged 30–38 years.[5] Participants underwent one unstimulated cycle, followed by one conventionally stimulated cycle. Embryo development was investigated using timelapse, and blastocyst quality was assessed using next generation sequencing based pre-implantation genetic testing. Results showed the mean number of oocytes required for one euploid blastocyst were not significantly different across both cycles (p=0.696). Bosch concluded: "You are not getting better eggs when you are doing a natural cycle; it's exactly the same." Similarly, the euploidy rate remained constant across both cycles.

"Conventional stimulation remains the gold standard."

Considering safety, Bosch highlighted that a lower incidence of OHSS was relevant 20 years ago. However, today we have tools that were not available before, namely gonadotropin-releasing hormone antagonist cycle triggering. The results of a meta-analysis show virtually no incidence of OHSS when treated with an agonist, with only two cases identified in a study using low doses of human chorionic gonadotropin for luteal phase support.[6]

Cost, the third cornerstone of mild stimulation, can be challenged when specifically considering cost-effectiveness. Bosch compared data from van Tilborg et al.[7] to data obtained from an age matched cohort at IVI Valencia, Spain, in the same period, permitting the comparison of mild and conventional stimulation and associated costs. The total cost was higher for conventional stimulation, due to increased dose volume and increased embryo numbers. However, when factoring live birth rates of 30.7% and 55.3% for mild and conventional stimulation, respectively, conventional stimulation was considered more economically efficient.

Bosch concluded that the three cornerstones of mild IVF, cost, safety, and increased oocyte quality, are not evident in practice. Thus, conventional stimulation remains the gold standard. However, Bosch acknowledged that there are exceptions to every rule, namely patients with a flat response to starting follicle-stimulating hormone, or patients with a high ovarian reserve. Increasing the dose in these patients is ineffective, with 'mini' ovarian stimulation more effective.

CONCLUDING REMARKS

Both speakers then addressed each other's presentations, challenging their presentations. Nargund focused on cost, stating that conventional IVF is a gold standard "for those who have gold," and re-emphasising the health economic evaluation discussed previously. Bosch concluded that the main cause of unsuccessful IVF is dropout, stating that females would prefer one cycle yielding 15 oocytes over two yielding eight. While three cycles are required with mild stimulation, 1.8 cycles are required with conventional, so simply more females achieve the end goal with conventional stimulation.

References

1. Datta AK et al. Mild versus conventional ovarian stimulation for IVF in poor, normal and hyper-responders: a systematic review and meta-analysis. Hum Reprod Update. 2021;27(2):229-53.
2. Fanton M et al. A higher number of oocytes retrieved is associated with an increase in fertilized oocytes, blastocysts, and cumulative live birth rates. Fertil Steril. 2023;119(5):762-9.
3. Polyzos N et al. Cumulative live birth rates according to the number of oocytes retrieved after the first ovarian stimulation for in vitro fertilization/intracytoplasmic sperm injection: a multicenter multinational analysis including ~15,000 women. Fertil Steril. 2018;110(4):661-70.
4. Baart E et al. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. Hum Reprod. 2007;22(4):980-8.
5. Labarta E. DuoStim: a new strategy proposed for women with poor ovarian response. Fertil Steril. 2020;113(1):76-7.
6. Youssef MAFM et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. Cochrane Database Syst Rev. 2014;DOI:10.1002/14651858.CD008046.pub4.
7. Van Tilborg TC et al. Individualized FSH dosing based on ovarian reserve testing in women starting IVF/ICSI: a multicentre trial and cost-effectiveness analysis. Human Reprod. 2017;32(12):2485-95.

Abstract Highlights

The following selected highlights draw attention to numerous interesting timely abstracts presented at the European Society of Human Reproduction and Embryology (ESHRE) 39th Annual Meeting, covering topics such as obstetric and neonatal outcomes following embryo biopsy, the role of genital tract infection and pelvic surgery in endometriosis, and the impact of fertility and preservation in patients with cancer.

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Cardiovascular Morbidity and Mortality in Females with Polycystic Ovary Syndrome

POLYCYSTIC ovary syndrome (PCOS) is strongly linked to cardiovascular risk factors. However, in females (aged 40–60 years), it does not heighten the risk of cardiovascular morbidity and mortality. PCOS is a common endocrinopathy in individuals of reproductive age, and can be characterised by anovulation, hyperandrogenism, and polycystic ovaries on ultrasound surveillance.

Females diagnosed with PCOS are at an elevated risk of developing adverse cardiometabolic outcomes, inclusive of Type 2 diabetes, metabolic syndrome, hypertension, and dyslipidaemia. These unfavourable risk factors would seemingly increase the risk of cardiovascular morbidity and mortality in these individuals. However, the evidence from epidemiological studies showcases a heterogeneous body of research with conflicting results.

At the European Society of Human Reproduction and Embryology (ESHRE) 39th Annual Meeting, held in Copenhagen, Denmark, Triada Doulgeraki, Obstetrics and Gynaecology, Royal London Hospital, UK, presented data from a cohort study. The study included a total of 75,142 participants from the UK Biobank, of whom 15,747 were diagnosed with PCOS. The participants were followed up for 11.1 years on average. Cox regression analysis was performed and adjusted for confounders as well as risk factors to quantify the risk of cardiovascular morbidity and mortality.

The study's primary outcome was the morbidity and mortality from ischaemic heart disease and stroke. The findings reveal that, in females with PCOS, the rate of cardiovascular events was 1.92 per 1,000 person-years, whereas the rate was 1.90 per 1,000 person-years in females without PCOS. It should be noted that PCOS was correlated with a heightened risk of obesity (odds ratio [OR]: 1.63; 95% confidence interval [CI]: 1.56–1.70), hypertension (OR: 1.18; 95% CI: 1.13–1.23), and Type 2 diabetes (OR: 1.44; 95% CI: 1.31–1.58).

"Females diagnosed with PCOS are at an elevated risk of developing adverse cardiometabolic outcomes."

The UK Biobank recruitment experienced a low response rate of 5.5%. Hence, the authors acknowledged that this may introduce a potential healthy responder bias, which may limit the representation of the population. Nonetheless, the findings highlight the need to strengthen public health strategies for surveillance, lifestyle interventions, and prompt treatment of comorbidities in females with PCOS.

Declining Sperm Motility Between 2017–2022 in Denmark

CHANGES in sperm quality have been assessed in several recent studies. However, while some studies report a decline in sperm quality, others dispute it due to potential biases in the populations studied, or differences in the methodological approach to investigating sperm quality. Resolution of these inconsistencies is critical due to the implications for human fertility, as well as for those involved in donor recruitment in medically assisted reproduction.

A research team, led by Robert Montgomerie, Queen's University, Kingston, Ontario, Canada, therefore sought to investigate the sperm quality among candidate sperm donors in Denmark between 2017–2022.

The semen quality of 6,774 candidate sperm donors attending Cryos International, Denmark, for their first semen analysis between 2017–2022 was analysed, regardless of whether the candidate was accepted as a donor. Four centres across Denmark were used to recruit participants aged 18–46 years. Ejaculates were examined within 1 hour of production. Specifically, semen volume was estimated by weight and sperm concentration, and the concentration of Grade A and B spermatozoa were measured across all years at each of the four sites. Data analyses were controlled for age, site, ejaculate volume, and average monthly temperature. Further analysis of longitudinal data was possible for accepted donors, allowing the research team to test for methodological biases.

Between 2017–2022, there was no evidence of changes in either semen volume (median: 3.5 mL) or sperm concentration (median: 58 million/mL) in the included population. However, there was a clear decline in the concentration and total number of Grade A and B motile sperm. The average concentration of Grade A sperm declined from 5.15 million/mL in 2018 to 3.33 million/mL in 2022. The same pattern was evident across the four test centres, but candidates from the city of Aarhus had lower overall sperm quality, measured as Grade A sperm motility. Longitudinal data from accepted repeat donors during the same period allowed the research team to rule out methodological factors that might have influenced these findings.

> **"There was a clear decline in the concentration and total number of Grade A and B motile sperm."**

Overall, there was no change in sperm concentration for candidate donors between 2017–2022. However, sperm quality declined by approximately 35% after controlling for age and other potential cofounders. Furthermore, this study suggests candidate sperm donors are a useful population in which to monitor changes in semen quality. The research team acknowledge, however, that they cannot rule out the possibility that males with poor sperm quality were more likely to apply to be donors. ●

Endometriosis: Do Genital Tract Infection and Pelvic Surgery Play a Role?

INFLAMMATION is considered to be a key contributor in endometriosis pathology. Both surgery and genital tract infections induce inflammation in the pelvis, and could therefore contribute to the pathogenesis of endometriosis.

Data from a retrospective cohort study evaluating whether endometriosis incidence was higher in females with a recent history of pelvic surgery and/or genital tract infection was presented at the European Society of Human Reproduction and Embryology (ESHRE) 39th Annual Meeting. Using data from the Korean National Health Insurance Service–National Sample Cohort I (KNHIS-SC I) from 2002–2013, researchers identified females aged 20–49 years who had received a diagnosis of genital tract infection, or underwent pelvic surgery between 2002–2008. Once identified, these patients were followed for 5 years.

A total of 34,018 females were identified and categorised into three groups: history of pelvic surgery (n=2,984), recent genital tract infection (n=30,336), and both pelvic surgery and recent genital tract infection (n=788). Comparison groups were matched for sociodemographic factors.

The analysis revealed that the incidence of endometriosis per 1,000 person-years in each case group was 5.37 for the recent genital tract infection group, 5.17 for the history of pelvic surgery group, and 20.81 for the group with both a history of recent genital tract infection and pelvic surgery. The incidence was significantly higher in case groups compared to comparison groups.

The adjusted hazard ratio for recent genital tract infection and endometriosis development was 2.29 (95% confidence interval: 1.99–2.63). Adjusted hazard ratios were also elevated for history of pelvic surgery at 2.10, and a history of both recent genital tract infection and pelvic surgery at 7.82, indicating that pelvic inflammation secondary to genital tract infection and pelvic surgery may contribute to endometriosis development.

These interesting findings highlight that appropriate treatment for genital tract infections and minimising tissue injury during surgical procedures may contribute to reducing the incidence of endometriosis. However, the authors noted several study limitations, including discrepancies between KNHIS diagnosis and treatment codes, and the clinical diagnosis and/or treatment; absence of information on diagnostic method; disease severity; and indication for pelvic surgery.

"The incidence was significantly higher in case groups compared to comparison groups."

Is Pregnancy Following Hormone Receptor-Positive Breast Cancer Safe?

PREGNANCY following treatment for hormone receptor-positive breast cancer is safe, according to research presented at the European Society of Human Reproduction and Embryology (ESHRE) 39th Annual Meeting. A common malignancy in females of childbearing age, patients and healthcare professionals are often concerned about potential detrimental effects of pregnancy following treatment.

However, several studies have demonstrated the safety of pregnancy after treatment, and the researchers searched for these using Medline, Embase, and Cochrane as part of a systematic literature search. There were no language or date restrictions up to 1st January 2023. Retrospective or prospective case studies were included, as well as cohort studies and prospective clinical trials that compared survival outcomes for patients who were pre-menopausal with reported pregnancy, or not after breast cancer treatment.

There were eight eligible studies, including 3,805 patients with hormone receptor-positive breast cancer. Of these, 1,285 had become pregnant after treatment. The median follow-up for all these studies ranged from 3.81–15.80 years.

Six of these studies reported overall survival, where patients who had become pregnant after breast cancer had a better overall survival compared with patients who did not (hazard ratio [HR]: 0.46; 95% confidence interval [CI]: 0.27–0.77; $p<0.05$). Three studies reported on disease-free survival, and no difference was observed in patients, regardless of subsequent pregnancies (HR: 0.96; 95% CI: 0.75–1.24; $p=0.781$).

Furthermore, no detrimental effect was seen in terms of disease-free survival in patients achieving a late pregnancy, which was defined as 2 or 5 years after breast cancer diagnosis, compared with patients who did not get pregnant post-treatment (HR: 0.63; 95% CI: 0.80–1.46; $p=0.611$). Patients who had an early pregnancy saw an increase in disease-free survival (HR: 0.63; 95% CI: 0.47–0.85; $p<0.05$).

However, there were some limitations. The meta-analysis consisted of abstracted data, with most studies being retrospective cohort studies. Furthermore, adjuvant hormone therapy was not available in many of the included studies. Despite this, these results do strengthen evidence that pregnancy after hormone receptor-positive breast cancer is safe.

"A common malignancy in females of childbearing age, patients and healthcare professionals are often concerned about potential detrimental effects."

Obstetric and Neonatal Outcomes Following Embryo Biopsy

RESEARCH presented at the European Society of Human Reproduction and Embryology (ESHRE) 39th Annual Meeting sought to investigate whether genetic testing of embryos pre-implantation was linked to adverse outcomes, both obstetric and neonatal. This practice has been used since 1990, to prevent transmission of diseases. It has evolved to include testing for couples who both carry the same autosomal recessive disorder, females experiencing recurrent miscarriage, and those at higher risk during pregnancy.

Currently, genetic testing of embryos pre-implantation is carried out at the blastocyst stage (5–7 days following fertilisation), along with the biopsy of a few trophectoderm cells from what would become the placenta, the transport of biopsy tissue to another site for genetic analysis, and the cryopreservation of blastocysts. Following this process, a single euploid embryo is transferred to the uterus as a frozen embryo.

"Currently, genetic testing of embryos pre-implantation is carried out at the blastocyst stage (5–7 days following fertilisation)."

A team from the University of Massachusetts' Chan School of Medicine, East Longmeadow, USA, compared the outcomes of frozen-thawed single embryo transfer with biopsy to frozen-thawed single embryo transfer without biopsy. The team linked birth certificates and maternal and neonatal hospitalisation discharge with surveillance data held on assisted reproductive technology in the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART-CORS). All data was reported across the state of Massachusetts between 2014–2017. Only singleton births following frozen-thawed single embryo transfer were included. Outcomes of cycles that underwent embryo biopsy (n=585) were compared to those with no biopsy (n=2,191). Comparison was adjusted for the mother's age, race, education, parity, birth year, previous infertility diagnoses, insurance, and BMI.

The study identified no differences between either cohort with regard to many outcomes, including pre-eclampsia, placental disorders, pregnancy-induced hypertension, low birthweight, Caesarean-section delivery, length of stay after birth for mother or baby, or gestational diabetes. Results were compared to four other studies, which focused on contemporary frozen-thawed embryo transfers, and were found to be consistent, with no effects observed on low birth weight, gestational diabetes, placenta previa, placenta accreta, and pregnancy-induced hypertension. The study concluded that the practice of genetic testing of embryos pre-implantation is generally safe, with regard to both maternal and neonatal outcomes.

Intra-Ovarian Injection of Platelet-Rich Plasma Does Not Improve Outcomes

INTRA-OVARIAN injection of autologous platelet-rich plasma (PRP) does not increase the oocyte yield number in young patients with poor ovarian response (POR), according to data presented at the the European Society of Human Reproduction and Embryology (ESHRE) 39th Annual Meeting. When it comes to controlled ovarian hyperstimulation (COH) for *in vitro* fertilisation (IVF), POR is a major hurdle. Several methods aiming to attempt follicular reactivation, such as intraovarian injection of autologous PRP, have shown promising results in prospective and retrospective cohort studies. A multicentre, randomised control trial evaluated whether PRP improved IVF outcomes in patients with POR.

"When it comes to controlled ovarian hyperstimulation (COH) for *in vitro* fertilisation (IVF), POR is a major hurdle."

In total, 83 patients were randomised to receive no intervention (control group; n=42), or autologous intra-ovarian PRP injection (PRP group; n=41) prior to COH. Participants were younger than 38 years, had two or more prior cycles with <3 oocytes retrieved, and did not have single gene disorders, endometriomas, prior ovarian surgery, severe male factor infertility, or BMI >35. After receiving treatment, participants underwent COH, oocyte retrieval, intracytoplasmic sperm injection, pre-implantation genetic testing for aneuploidy, and single frozen euploid embryo transfer. The primary outcome was number of MII oocytes obtained, and secondary outcomes included sustained implantation, blastocyst and euploid blastocysts yield, and ovarian reserve tests.

The team did not note any significant differences in number of MII oocytes retrieved (3.1±3.3 versus 2.8±2.4 in PRP versus control, respectively; p=0.9), euploid blastocysts (0.9±1.6 versus 0.8±1.1; p=0.5), or blastocysts (1.3±2.1 versus 1.0±1.3; p=0.8) per cycle. Furthermore, there were no differences in the rate of sustained implantation (29% versus 31%; p=0.9; relative risk: 1.0; 95% confidence interval: 0.7–1.3) or the likelihood of obtaining at least one euploid blastocyst (37% versus 45%; p=0.4; relative risk: 0.9; 95% confidence interval: 0.6–1.2).

Limitations of the study included the lack of evaluation of long-term effects due to oocyte retrieval taking place in the cycle immediately following treatment. The team concluded that intra-ovarian PRP did not improve outcomes, and they therefore do not support the wide utilisation of PRP for IVF in patients with POR. ●

The Impact of Fertility Preservation in Patients with Cancer

TECHNIQUES used to preserve fertility in females with cancer include oocyte vitrification (OV), ovarian cortex cryopreservation (OCC), and embryo vitrification. Whilst these techniques have become a pivotal part of cancer care, data on their impact on ovarian damage, pregnancy outcomes, disease relapse, and survival is limited.

Researchers from the Research Health Institute La Fe, Reproductive Medicine Research Group, and IVI Foundation, Valencia, Spain, performed a prospective cohort study to investigate this further. They enrolled 695 patients referred to fertility preservation (FP) units in two hospitals between 2001–2016. Patients were followed up for at least 5 years from time of enrolment. The primary outcome was median survival after FP. Usage rate of FP, relapse rate, premature ovarian insufficiency (POI), poor ovarian response, clinical pregnancy, and live birth rates were secondary outcomes.

Of the 695 enrolled, 556 received OV, OCC, or embryo vitrification. The remaining 139 patients received no FP treatment. Overall, the study found that treatment resulted in ovarian damage in almost half of patients, and natural live birth was achieved in approximately one-third of those with a pregnancy wish.

There was no significant difference in survival between those who received FP and those who did not (median survival time of 89.67 months and 92.81 months, respectively; p=0.3). However, a significant survival difference was seen when comparing patients who had received approval to get pregnant (98.84 months) to those who had not (84.79 months; p<0.001). No difference was seen between patients with hormone-dependent breast cancer undergoing ovarian stimulation for OV versus OCC at 95.62 months and 87.38 months, respectively (p=0.37).

Ovarian damage occurred in 334 patients (48.06%), and the incidence of POI was 20.29%. Patients with POI were of significantly increased age (p<0.001), and had received high-risk chemotherapy more frequently (p<0.001). Cryopreserved material was used by 86 patients.

"There was no significant difference in survival between those who received FP and those who did not."

Spontaneous live birth occurred in 84/266 patients with a pregnancy wish (31.58%). Those able to conceive naturally were significantly younger at 30.71 years compared to 33.46 years for those unable to conceive naturally (p<0.001), and more frequently received low-risk chemotherapy (p=0.018). Live births occurred in 37/86 patients after use of FP.

The authors noted that the higher survival seen in patients who used their cryopreserved material was mediated by disease prognosis, which limits pregnancy chance to those with stable disease, and concluded that even when ovarian stimulation is used, fertility preservation does not negatively impact survival. ●

Plasma Rich in Growth Factors Treatment Produces Improved Reproductive Outcomes

ENDOMETRIAL plasma rich in growth factors (PRGF) therapy produced interesting reproductive outcomes in patients with thin endometrium (ThE), recurrent implantation failure (RIF), and recurrent miscarriage (RM). Researchers in Alicante, Spain, observed significantly increased reproductive success rates with PRGF in patients with ThE and RIF, and unaffected results for pregnancy loss in the RM group. The findings from this study were presented at the European Society of Human Reproduction and Embryology (ESHRE) 39th Annual Meeting.

Use of PRGF has been successfully applied in other medical fields, but has become a novel treatment method in reproductive medicine. The current research investigated its usefulness in ovarian follicle activation and enhancing endometrial receptivity. This research was a retrospective analysis from 2016–2022, including 107 patients recruited into ThE (n=64), RIF (n=36), and RM (n=7) groups.

Live birth and ongoing pregnancy rates per embryo transfer were compared with success rates obtained in previous embryo transfers that preceded PRGF intervention. The total of 107 patients underwent 150 endometrial PRGF treatments and 131 embryo transfers. Altogether, 19 embryo transfers were cancelled, higher in the ThE group than RIF (16% versus 7%). In both the ThE and RIF cohorts, positive pregnancy, clinical pregnancy, and ongoing pregnancy/live birth rates per embryo transfer were significantly higher with PGRF treatment compared with previous embryo transfer. Meanwhile the RM group showed no significant difference for these measures, and no ongoing pregnancies were achieved. To date, 20 singletons and one set of twins have been confirmed to be born from the PRGF cycles involved in this study, with 12 more pregnancies still ongoing.

The tangible results from endometrial PRGF that are reported show real promise for application in reproductive medicine; however, the researchers did report several limitations. Patients with RM were too few in number to evaluate pregnancy loss rates, and the self-controlled design of study may have influenced the comparison between pre- and post-intervention pregnancy rates. Heterogeneity of clinical severity between included patients with ThE could also affect observed reproductive outcomes. Regardless, this study has presented that regenerative therapy using PRGF is a safe, affordable, and efficient treatment option for patients with ThE and RIF. ●

"The total of 107 patients underwent 150 endometrial PRGF treatments and 131 embryo transfers."

Interviews

Stephen Franks, Diana Kuh, and Søren Ziebe spoke with EMJ, sharing details about their careers and research focuses. The experts also discussed a range of field specific topics, including polycystic ovary syndrome, life course epidemiology, and assisted reproductive technology.

Featuring: Stephen Franks, Diana Kuh, and Søren Ziebe

Stephen Franks

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Q1 What led you to pursue a career in reproductive health? Was there a specific event or person that was particularly influential?

It was by accident really. I was very keen to have a career in internal medicine and did not really know what specialty to follow. I flirted with the idea of cardiology but that is a bit too exciting really. When I was looking for a training job in internal medicine, and not having a lot of success, my oldest friend from medical school said their brother, Howard Jacobs, was looking for somebody to do some research in endocrinology. He was doing this wonderful project on measuring prolactin in females with primary amenorrhea. We applied for a fellowship, which we got from the Royal College of Physicians. Although I had not really thought about doing lab research, I really enjoyed that. We set up an assay for prolactin, which in those days was not very easy. I carried on training and did a postdoc in Canada in reproductive endocrinology. When I came back, I continued my training in general endocrinology and research in reproductive endocrinology, which I still have an interest in. I then started a position as a senior lecturer of reproductive endocrinology, in the Department of Obstetrics and Gynaecology, in what was then St. Mary's Hospital, London, UK, which has since become part of Imperial College. So, I got bitten by the bug of research and endocrinology, particularly reproductive endocrinology, and the rest is history.

> **"The big gaps are in female health, and there is still a lot to be learnt about the effect of the environment on reproduction."**

Q2 You currently have more than 500 international publications to your name for your research in endocrinology and reproductive biology. What do you believe to be the current gaps in literature and which topics merit greater attention?

I think the big gaps are in female health, and there is still a lot to be learnt about the effect of the environment on reproduction and how obesity affects reproduction. We have lots of new information about neuroendocrinology of reproduction, but there is still more to learn there, such as how to manage menopausal symptoms.

Q3 Over the last 10 years what have been the most significant advances within the field of reproductive endocrinology?

We have learnt about the brain and reproduction, particularly neuropeptides, and there have been a lot of advances on these in the last 10 years. Kisspeptin for example, which is a neuropeptide in the hypothalamus, translates environmental signals and conveys them to the gonadotropin-releasing hormone (GNRH) neurons. Those do not have steroid receptors themselves, but kisspeptin neurones do. So, it is like a master controller of GNRH. In the last 10 years, a lot has been learnt about its physiology, as well as therapeutic implications. For example, whether you could give kisspeptin to females who have hypothalamic amenorrhea to get them to ovulate. The results look promising, although they are not defined yet. Waljit Dhillo's group has looked at neurokinin B and its receptor, and discovered that if you block the neurokinin B receptor, you can stop menopausal flushes. This is an advance in the management of menopause, which is very significant. So, for females who cannot take oestrogen, it looks like an extremely promising treatment, and in fact I think it is now licensed for management. That is an example of how researching neuropeptides helps, and we have also learnt a lot more about what controls gonadotrophin secretion in polycystic ovary syndrome (PCOS).

> **"The ignorance about the implications of polycystic ovaries is still there and while it is getting better, it is not good enough."**

Q4 Your recent research specifically focuses on PCOS. Are there any advances you are particularly excited by in this field?

Something I think has advanced considerably in the last 10 years, relating to PCOS, is genetics of reproductive endocrine disorders. For example, in hypothalamic amenorrhea, genes that are related to GNRH secretion have been identified. Even in females with functional hypothalamic amenorrhea, who are underweight or exercise a lot, and whose period is switched off, it seems that there can be a genetic predisposition to losing their periods as a result of impaired gonadotrophin secretion. In the field of PCOS, genetics have been very important and have led to some advances. What we have learnt about PCOS is how important it is in terms of long-term health and particularly mental health. It is a significant factor in terms of anxiety and depression, as well as quality of life issues, which are more likely in females who suffer from hirsutism and have to shave daily. But even in those who do not have such severe symptoms, it is a visible problem that we have learnt from population studies that I have been involved in over 20 years. We have been able to look at females born in 1966 and 1986 and followed them through until quite recently. This has given us a bit more insight into what impact infertility has in PCOS. We can also look at the impact of obesity over the years in females with PCOS. So, I think that has been a very important advance for us.

Q5 You are due to present on 'The different phenotypes of PCOS: Implications for clinical practice' at ESHRE 2023. How widespread do you believe the knowledge of PCOS is and are there any misconceptions?

The answer is the knowledge is not widespread enough. While there is more knowledge amongst general practitioners or even specialists such as gynaecologists and endocrinologists, there is not enough. I would still get patients referred to me who have had a scan because they had pelvic pain, found they had polycystic ovaries, and were told they will not be able to get pregnant and should think about adoption. The ignorance about the implications of polycystic ovaries is still there and while it is getting better, it is not good enough. There are initiatives around the

globe to try and improve awareness, and I am involved with a patient support group called Verity, which tries to get proper information to outpatients. Rachel Hawkes, the chair of Verity, has been involved in developing the international guidelines for management of PCOS. So, that is a way of improving awareness amongst the public and therefore amongst GPs, at the same time encouraging patient empowerment.

Q6 In the recently published review you co-authored, entitled 'Obesity and Polycystic Ovary Syndrome'. What was the key message you were trying to deliver?

What is interesting is that females with PCOS seem to be predisposed to obesity, but it is a vicious circle. If you get obese, your symptoms get worse, and if you lose weight, you get a lot

better; however, that is not so easy to do. One of the things we are very interested in, is looking at the possibility of an energy balance in females with PCOS. This follows up on a paper we published 30 years ago, where we showed that energy expenditure after a meal, so postprandial energy expenditure, is reduced in females with polycystic ovaries; however, there was no difference in resting energy expenditure. I later worked with Colin Duncan, who wanted to try to determine if the defect in postprandial thermogenesis was androgen-dependent. He has a sheep model of PCOS, which equals a clinically credible model, because sheep do have all the features of PCOS, such as excess hair, as well as reproductive and metabolic features of PCOS, if they are exposed to androgen in late foetal life. We tried to determine whether these were androgenised sheep and whether they had this disorder of energy panels, and the answer is they do. Duncan went on to look at whether you could actually reverse that. He had this very smart idea of giving the animals intranasal insulin, because it is related to insulin insensitivity, and he was able to show that you could reverse the postprandial defect in these prenatally androgenised sheep. I am now a collaborator on a grant he got to see whether intranasal insulin in females improves energy expenditure.

Q7 You have chaired the Society for Endocrinology, you were president of the Section of Endocrinology and Diabetes of the Royal Society of Medicine (RSM), and hold an honorary doctorate from the University of Uppsala, Sweden. What achievement are you most proud of?

I suppose passing on a passion for reproductive endocrinology to people who have trained with me. There are lots of things that I feel quite proud of in terms of research, for example the idea that we might be following up on this disorder of energy balance. I think I was one of the first to suggest that a weight loss of a 5–10% made a big difference to resuming ovulation. I was also involved in the early studies looking at prevalence of polycystic ovaries in collaboration with Judith Adams in 1986. She could see polycystic ovaries on ultrasound, in days when it was not easy to do that. So, that made a difference because we could look at different patient groups, which revised the idea of how prevalent it was.

Q8 As an educator, where can we expect to see your focus lie in the field of reproductive health over the coming years?

I have been practising for a very long time, so I am thinking about winding down. I have already finished my clinical work and while I miss my patients, I do not regret having given up. So, my focus is on research at the moment. From a very personal point of view, I am looking at thecal cells in females with PCOS, because not a lot is known about them. For example, more knowledge is accumulating about where the thecal cells come from and what signals are involved. That is something we would like to pursue in collaboration with people in Cambridge and Copenhagen, where they have an ovarian cryopreservation programme. For females who are about to have cancer treatment, which is likely to make them infertile, they offer the possibility of removing one ovary. If the cancer treatment is not ablative, in terms of reproductive function, they have a functioning ovary. The other ovary is cut up and little pieces of cortex, which contain the primordial follicles and early growing follicles, are frozen. They can then be auto transplanted and there have now been one or two pregnancies. When they cut out the medullary tissue just below the cortex, it contains lots of follicles unsuitable for freezing, so we can get small ovarian follicles of different sizes, which we can get thecal cells from. That is our source and we are lucky to have that collaboration. So, I am looking forward to that; we put in a grant application and we will see.

Diana Kuh

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Q1 What led you to pursue a career focusing on life course epidemiology?

After leaving Cambridge University, UK, with a degree in economics, in 1975 I became a research scientist at Exeter University, Devon, UK. I first joined the Operational Research Unit, where I used mainframe computer models to help allocate healthcare funds, which was sometimes frustrating. I then joined the Paediatric Research Unit, studying the unmet needs of adolescents and young adults with disabilities, which was a big learning experience. There, I heard about the Medical Research Council (MRC) National Survey of Health and Development (NSHD), the oldest of the British birth cohort studies, following 5,362 participants since their birth in March 1946. I was lucky enough to become an MRC scientist on that birth cohort study at University College London (UCL), UK, in 1987, and eventually the Scientific Director of the study, and Founding Director of the MRC Unit for Lifelong Health and Ageing from 2007–2017.

One of the first analyses I undertook was for a paper led by the late Professor David Barker, at a time when there was a revival in the early origins of cardiovascular and respiratory diseases. David's hypothesis focused on exposures in foetal life. It was a wonderful period for using data from birth cohort studies to investigate this intriguing hypothesis. This became the focus of my PhD and various articles, which turned me into an epidemiologist. With Yoav Ben-Shlomo, I co-edited 'A Life Course Approach to Chronic Disease Epidemiology',[1,2] persuading our colleagues to contribute chapters reviewing the evidence for lifetime risk factors on later life chronic diseases. This book was the founding text for life course epidemiology, which studies how biological, psychological, and social factors across life affect adult health, ageing, and chronic disease risk. This led to a series of life course books (I co-edited one on women's health in 2002 and one on healthy ageing in 2014), as well as over 500 peer-reviewed articles. The second edition of 'A Life Course Approach to Women's Health', co-edited with Gita Mishra and Rebecca Hardy, is due out in July 2023, and a third edition of 'A Life Course Approach to The Epidemiology of Chronic Diseases and Ageing' has just begun!

Q2 As a world-leading pioneer within the field of public health, what initially sparked your specific interest in women's health?

The study members were 41 years old when I joined the NSHD research team, and I saw an opportunity to ask women participants about their gynaecological health in 1989, at age 43, when nurses interviewed them at home. Back then, there were few longitudinal studies of the menopausal transition, and none with prospective data from birth, so with Rebecca Hardy, and later with Rachel Cooper and Gita Mishra, we sent out postal questionnaires every year between ages 47–54 years to capture the timing of perimenopause and natural or surgical menopause, symptoms, and healthcare, including a full hormone replacement therapy history. Because of the high response rates, we were able to look first at a range of lifetime risk factors for timing of menopause, and

other related experiences. We showed how early life exposures, such as better childhood socioeconomic circumstances, higher childhood cognitive ability, and breastfeeding, were associated with a later menopause, even after accounting for adult risk factors. Once we had collected further data at 60–64 and 69 years, we then showed how the menopausal transition was associated with later health, such as adult bone density, and cognitive and cardiovascular function.[3-5]

Q3 Focusing on menopause, do you believe healthcare professionals lack awareness, and what can be done to combat this?

Not all health professionals have sufficient training in, or time to devote to, the menopausal transition, so some may lack awareness of the full range of experiences associated with the transition and its implications. For example, they may not recognise the symptoms presented by women in their early 40s as being consistent with early menopause, or that some women may still experience vasomotor symptoms for more than a decade post-menopause. Women from culturally and linguistically diverse backgrounds may face difficulties in describing or expressing their menopausal symptoms, such as sexual difficulties caused by vaginal dryness. Well informed general practitioners and staff at menopause clinics, where they exist, can make all the difference to women's healthcare. We look forward to assessments of the new 'women's health hubs' proposed by NHS England, as currently there are insufficient services for all aspects of women's reproductive life.

Q4 Could you briefly summarise the main findings of the paper you co-authored, entitled, 'Is there a link between infertility, miscarriage, stillbirth, and premature or early menopause? Results from pooled analyses of 9 cohort studies.'?

This paper was led by Chen Liang and Gita Mishra as part of Gita's successful InterLace consortium, which has harmonised data from women participating in cohort studies worldwide (including NSHD), so has the power to investigate premature menopause (<40 years) and early menopause (40–44 years). In a pooled analysis of nine cohort studies, including 303,594 postmenopausal women, the median age at natural menopause was 50.0 years (interquartile range: 47.0–52.0). The percentages of women with premature and early menopause were 2.1% and 8.4%, respectively. The relative risk ratios (95% confidence interval) of premature and early menopause were 2.72 (1.77–4.17) and 1.42 (1.15–1.74) for patients with infertility; 1.31 (1.08–1.59) and 1.37 (1.14¬–1.65) for women with recurrent miscarriages; and 1.54 (1.52–1.56) and 1.39 (1.35–1.43) for women with recurrent stillbirths. The risks for Asian women with these histories were higher. The paper discusses the possible biological mechanisms underlying these associations, and suggests that women with such a reproductive history would benefit from early care and advice about its implications for menopause timing.

Q5 You are due to publish a second edition of your book, entitled, 'A Life Course Approach to Women's Health'.[6] What can the reader expect to learn?

I co-led the first edition with Rebecca, and Gita is the leading editor of the second edition, which we are excited to see published in July. The second edition updates and reviews the tremendous amount of new research evidence and advances in life course methods, examining the long-term influence of foetal and childhood experience in the development of chronic health conditions. We focus on conditions that are common or unique to women, and that typically impact physical and mental health in midlife and beyond. There are new chapters on lifetime factors associated with endometriosis and lung function in women; on the role of integrative omics in understanding chronic conditions; and on the impact of violence on women's long-term health. The chapter on knowledge translation describes how we can translate all the epidemiologic evidence to inform better health policy, practice, and promotion. This is particularly important, as the life course approach is increasingly adopted around the world as a framework for developing national strategies for women's health.

Q6 You have also applied your career in life course epidemiology to ageing and cognitive development, establishing and directing the MRC Unit for Lifelong Health and Ageing, and acting as Scientific Director of the MRC NSHD. What have been the most exciting developments in this field in recent years?

There have been many exciting developments, and I give an example in my response to question 7. Increasingly, life course and ageing researchers can use repeated measures within a cohort study to investigate different aspects of functional change at the individual, and increasingly the system and cellular levels, and identify lifetime risk factors, mediators, modifiers, and consequences of functional trajectories. Cross-cohort studies have proliferated in recent years, enabling, data permitting, the whole life course to be investigated, to check whether findings are replicated, and vary by age or birth cohort. Intensive biology is providing multiple omics measures, and the relatively new field of exposomics is providing more detailed environmental exposures over time, to incorporate into cohort studies. Advances in life course and longitudinal methods are being used to analyse these data. The COVID-19 pandemic accelerated the development and use of remote data capture within cohort studies, which will persist, but needs to be carefully evaluated. More cohort studies are taking place in middle- and low-income countries, and increasingly the challenge is to translate the growing body of evidence into intervention studies to improve population health, as well as into practice and policy-relevant guidelines.

Q7 Returning to the NSHD birth cohort study, what have been the most interesting findings from the 1946 cohort?

Overall, the key message from the thousands of papers based on NSHD is that childhood matters for adult health, emphasising the importance of each new generation having the best start to life. In broad terms, we showed that early socioeconomic disadvantage; poor childhood physical, cognitive, and emotional development; and prior ill-health adversely affected musculoskeletal, cardiometabolic, cognitive, and reproductive function in midlife and long-term survival, even accounting for adult lifestyle and socioeconomic conditions. In turn, midlife functional performance identified individuals, before symptoms were manifesting, who had a higher risk of chronic disorders in later life. Repeat measures of function across adult life showed, in some cases, that early life disadvantage also affected functional change, and led to accelerated ageing. Identifying individuals at risk earlier would enable timely preventive action to be undertaken.

Taking musculoskeletal ageing as a specific example, in the 1990s, my colleagues and I published papers showing that patterns of physical growth, motor and cognitive development, and childhood adversity were differentially associated with grip strength and other measures of physical performance at age 53 years. Two decades later, after two more assessments of grip strength, we showed that parameters of physical growth, such as birthweight and height tempo; attainment of motor milestones; and childhood cognitive ability remained persistently associated with adult grip strength, and that higher childhood cognitive ability was also associated with a slower decline

in strength, even after taking account of adult factors.[7] Then, after collecting intermediate markers of heart and kidney damage in midlife, we went on to show that lower levels of N-terminal pro-B-type natriuretic peptide and IL-6 were independently associated with better physical performance up to 9 years later.[8] The associations were meaningfully stronger than those observed for conventional risk markers, including lipids, blood pressure, and glycaemia, and were not explained by the onset of cardiovascular and kidney disease or diabetes. Findings like these help design interventions across life to maximise muscle development and maintain strength as people grow older.

Q8 Your research paper, entitled, 'Worldwide Trends in Body-Mass Index, Underweight, Overweight, and Obesity from 1975 to 2016' is considered to be among your most cited publications. Why do you believe it was so impactful?

This paper, by the NCD Risk Factor Collaboration, is an excellent example of team science. It pooled together 2,416 population-based studies with measured height and weight data on almost 130 million participants from 1975–2016. I was privileged to be part of that collaboration, having contributed NSHD data, and along with all the other co-authors, I had the opportunity to comment on the draft manuscripts. It has been heavily cited because in children and adolescents (5–19 years), the sophisticated global analysis shows rising trends in mean BMI and in prevalence of obesity over 42 years in many regions of the world, even as underweight remains stubbornly high in some regions.

Of my other publications where I made a more substantial contribution, I am delighted that our original life course book,[1,2] the editorial on life course epidemiology with Yoav Ben-Shlomo,[9] and the glossary of life course epidemiology[10] remain highly cited.

Q9 Are there any publications or innovations on the horizon within the field of life course epidemiology that you are particularly excited about?

I was recently commissioned to do a third edition of our original life course book with Yoav Ben-Shlomo, Ezra Susser, and Joanna Blodgett as co-editors. This third edition of the life course book, two decades since the second edition, and a quarter of a century since the first edition, includes new chapters that address the value of life course epidemiology in the context of increasingly urgent global challenges, such as pandemics and climate change. Jo and I have a chapter on the life course and COVID-19 coming out soon, and we will develop our ideas further in the third edition. We have a wonderful group of 52 contributors who will bring together climate and life course epidemiology; assess recent developments, including COVID-19 and other contextual factors, that shape birth cohorts and other life course studies; review new life course research for specific chronic diseases, multimorbidity, and the underlying mechanisms of ageing; and address how a life course approach informs what can be done, and is being done, in low-, middle-, and high-income countries to develop policies and interventions to improve population health. I feel very privileged to work with so many wonderful colleagues who are experts in their fields.

References

1. Kuh D, Ben-Shlomo Y (eds.), A Life Course Approach to Chronic Disease Epidemiology (1997) 1st edition, Oxford: Oxford University Press.
2. Kuh D, Ben-Shlomo Y (eds.), A Life Course Approach to Chronic Disease Epidemiology (2004) 2nd edition, Oxford: Oxford University Press.
3. Kuh D et al. Age at menopause and lifetime cognition: findings from a British birth cohort study. Neurology. 2018;90(19):e1673-81.
4. Kuh D et al. Menopause, reproductive life, hormone replacement therapy and bone phenotype at age 60-64: a British birth cohort. J Clin Endocrinol Metab. 2016;101(10):3827-37.
5. O'Keeffe LM et al. Age at period cessation and cardiometabolic risk factors from 53 to 69 years: a British birth cohort study. Heart. 2020;106:499-505.
6. Kuh D, Hardy R (eds.), A Life Course Approach to Women's Health (2002) 1st edition, Oxford: Oxford University Press.
7. Kuh D et al. Developmental factors associated with decline in grip strength from midlife to early old age: a British birth cohort study. BMJ Open. 2019;9:e025755.
8. Kuh D et al. Systemic inflammation and organ damage in middle age are associated with physical capability in old age: findings from a British birth cohort study. Circulation. 2019;139:1988-99.
9. Ben-Shlomo, Y, Kuh, D. A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. Int J Epidemiol. 2002;31:285-93.
10. Kuh, D et al. Life course epidemiology. J Epidemiol Community Health. 2003;57:778-83.

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Q1 With over 20 years of experience in the field of reproductive medicine, what initially sparked your interest to pursue a career in this field and has motivated you to continue researching?

Honestly, it was kind of random. I was at a lecture talking about the mating behaviour of fish, and that triggered me to start speculating on how implantation happened in humans. This is the real reason why I got into reproductive medicine. On top of that, this was a very fascinating area. When I started, there was no, such thing as fertility clinics and assisted reproduction. The first child had been born through assisted reproductive technology (ART), but the general knowledge was not out there yet.

I educated as a biologist, and I had no idea that this was a career option and I accidentally found out. Then I was fascinated because it was a completely new field, and it was a field that combined both medicine and biology. There are lots of things we did not know and still do not know. We have to deal with three patients; we have an embryo, we have a female, and we have a partner; so, this was fascinating, and I got very intrigued by it.

Q2 You currently have more than 100 international publications to your name. What do you believe to be the current gaps in literature and which topics merit greater attention?

Over the years, we have worked very hard to look into stimulating oocytes, specifically endocrinology and understanding all the hormones: what they do, what they do not do, and what they work with. We have also researched oocyte and sperm biology, and which factors indicate developmental competence, so that we can select the embryo with the highest chance of pregnancy.

However, there is a huge area that we need to address where we have failed completely all over the world. This contrasts with what many of my colleagues here, at the University Hospital Copenhagen in Denmark, and other places do, which is working towards preventing diseases that we are very good at treating. Nobody has worked in preventing reproductive diseases, which I think is a huge problem and I started focusing on this more than 10 years ago. I tried to legitimise the debate addressing when we should have children, and how our biology is without being perceived as pressing young people, which is very difficult. But we need to have that debate. Here in Denmark, more than 12%

"Embryologists have very restricted access to material that we can use for research. We can have access to human sperm cells, but we don't have access to human oocytes."

of all children born through ART never become parents, and one in five males in Denmark never become a father. We set records every year in number of fertility treatments, illustrating that there is a wish to have children out there, but a lot of people miss their own biological window by postponing for many reasons; therefore, we need to start addressing the prevention of reproductive diseases, and how we ensure that the general population have an increased knowledge about reproductive biology.

Q3 In the recently published paper you co-authored, entitled 'A qualitative study on couples' attitudes and concerns regarding a freeze all strategy in ART treatment', what was the key message you were trying to deliver?

It is fantastic that we do studies now on couples' attitudes and concerns, so we are actively engaging in debate with our patients. How do they see things? This study showed a couple of things; the basic one was increased safety. Can we freeze all the embryos? If there is a risk of over stimulating the female, how do we ensure that, with the fantastic improvements in cryopreservation, the patients do not see this as a negative thing but understand that it is part of their safety.

Also, we found that starting treatment for patients is a relief. These are patients who have been trying to have a child for many years but failed. We are sort of a last stop, the last chance. Then when we start treating them and they feel a relief that now, finally, they are starting the process. However, they accept the safety of this if we inform them prior to starting treatment. If, for example, we say: "If you respond this way, we might either transfer the fresh embryos or cryopreserve them and transfer them in a later cycle, in order for us to ensure your safety."

Q4 You have specialised experience as a Senior Clinical Embryologist. What are some of the unique challenges associated with work in this field?

Embryologists have very restricted access to material that we can use for research. We can have access to human sperm cells, but we don't have access to human oocytes. So, we have to work around this problem, which very difficult is probably why some areas within embryology have developed very slowly.

Secondly, the title Senior Clinical Embryologist refers to a European Society of Human Reproduction and Embryology (ESHRE) initiative, which I was part of commencing. In many countries, including my country, you cannot study embryology. Maybe you can now some places, but in the last 20 years you have not been able to do that. Therefore, we needed to ensure that people working in this area were competent and had the best education they could have to fulfil this role. That is why we started this ESHRE initiative. It has been very successful in many ways and has increased knowledge tremendously in many groups working in laboratories. In recent years, it has also covered nurses and reproductive nurses so they can get a certification. They are studying a lot of the issues that we are working on and passing an exam that gives them the accreditation.

Q5 You co-founded the ESHRE 'International Fertility Education Initiative'. What are the main goals of this initiative?

I could talk about that for hours because I think that it is extremely important. When we talk about preventing diseases and educating the general population, it is important to realise that it is not about your fertility or my fertility, it is about our fertility. It takes two to have children. As a young female, you could be fertile, but if you fall in love with a male with no sperm cells you will not have children, and vice versa. Therefore, we need to discuss these things.

We know that the general awareness about these things is close to zero. While a lot of people think that they know something, most of that is wrong. We need to start addressing this to allow young people to make informed choices for their own life, and this means sex education. It is controversial to call it sex education in many places as it is not about sex, it is about reproduction. If a young male does not know the reproductive biology of their female partner, they might not be committing to having a family until it is too late for the partner to have children.

We need to prioritise this understanding. I call it reproductive sustainability and we need to prioritise this on a society level. We have very scary examples right now from South Korea, Japan, and Norway, where our societies are on the brink of collapse because we have too few children to maintain a well-functioning society. So, I think that it is very important to talk about these things.

"We need to start addressing this to allow young people to make informed choices for their own life, and this means sex education."

Q6 Over the years that you have been working in the field of assisted reproduction, how have you seen the field advance in terms of the technology used?

When I started in this area, we were only treating couples where the males had normal sperm quality and the females had blocked tubes and mechanical problems. However, we have achieved a lot within the profession since then; some say that we have reinvested all the gain that we have had to start treating other types of patients. When a great clinic in Brussels, Belgium, developed intracytoplasmic sperm injection, we could suddenly treat couples where the males had a low sperm quality.

We have had a lot of development, so that we can now treat, not only infertility problems, but also other types of problems such as young females with cancer. We can cryopreserve their ovarian tissue here at the clinic, not because they are having fertility problems but because the cure for that cancer will kill the oocytes in the ovary. We have couples who experience recurrent pregnancy loss. We have testing for genetic diseases. We are treating patients with HIV and hepatitis. We are treating so many different conditions that we could not dream of in the early days.

Q7 Since your appointment as Head of the Fertility Department at the Juliane Marie Centre (JMC) – Rigshospitalet, Copenhagen University Hospital, Denmark, what has been your proudest achievement?

It is the team that we have today, but I would like to stress that this is not my achievement: this is our achievement. It is how we have reached a point where we have crossed professional collaborations; we have very intense dialogue between doctors and biotechnologists, nurses, secretaries, and all the people who are needed to provide good professional services for the patient. Also, we recognise that many different professions are needed and are paramount to the research and success of what we do and what we try to achieve. I think that facilitating this way of working and seeing each other is fantastic change. However, I want to stress this is not my achievement: this is our achievement.

Q8 What advice would you give a young clinician trying to pursue a career in assisted reproduction?

I would give the same advice whether they were a young clinician, biologist, nurse, embryologist, secretary, geneticist, psychologist, or whoever is needed or has an important role to fulfil in assisted reproduction, and that is to go in and engage wholeheartedly. It is a very young area; it was only 40–50 years ago that the first child was born through. We still have so much to do and the demand for fertility treatments has increased enormously over the years. So, get in there, work wholeheartedly and enthusiastically, and engage in the work of preventing the problems that we have to treat as much as we do. One in eight children born through ART is way too high and we need to change that. And just imagine having a job that assists people in having a family. What a privilege!

The Menopause: Symptoms and Breaking the Stigma

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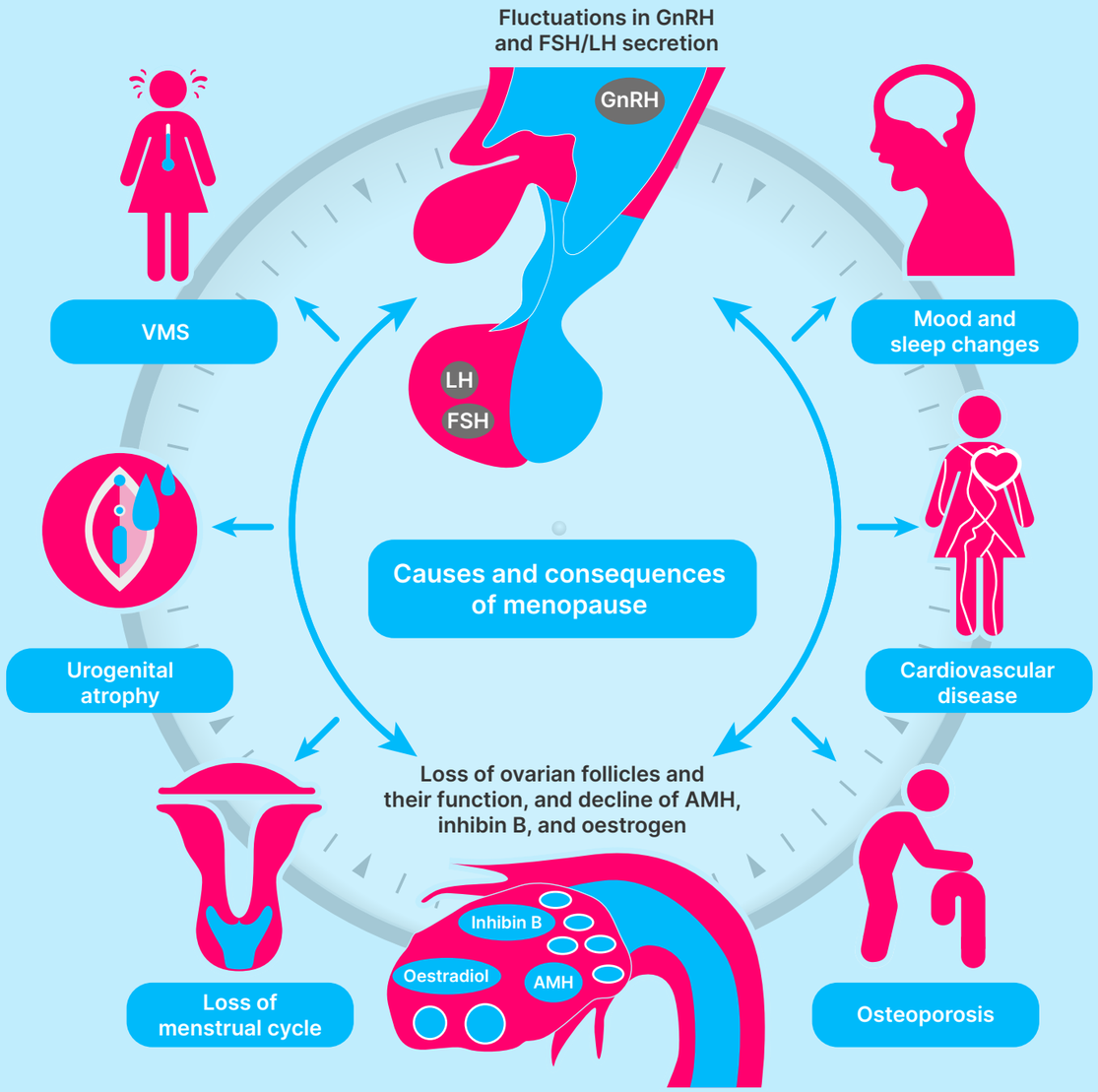
Symptoms

12 months after an individual's last menstrual period

A loss of ovarian follicular function

It occurs at the median age of **51**

Premature menopause can be indicated by an FSH level of 25 IU/L on two occasions



Mood and sleep changes: **74%** of females in Europe reported feeling tired or worn out as the most common menopausal symptom in a week

CVD: associated with an increased CVD risk in females aged **>55 years**

Osteoporosis: the average decrease in bone mineral density during the menopause is approximately **10%**

4.57* Weight gain is ranked as the most bothersome symptom of the menopause in Europe

(*with a score of **6.00** being extremely bothersome)

VMS

40% of females in five European countries said VMS were a moderate-to-severe problem

Frequency ranges from one a day to one an hour. The median duration of VMS is **7.4** years

Associated with poor sleep and a depressed mood, resulting in fatigue, irritability, forgetfulness, and decreased work productivity

Between **37-52%** of participants treated their hot flashes with over-the-counter products

In a study of 2035 post-menopausal females:

Hot flashes had a greater impact on daily activities than on work activities

On average, females experienced 4.6 hot flashes per day

Prevalence of VMS: 31% (France) 52% (Italy)

The proportion of females who have contacted a HCP in the previous 12 months to discuss VMS: 27% (UK) 64% (Italy)

Breaking the Stigma

On average, **64%** of females felt comfortable discussing the menopause with friends.

This ranged from **78%** (UK) to **<30%** (Hungary)

An observational study of 829 post-menopausal females:

- **49.0%** felt "not informed at all" prior to the menopause
- **51.1%** females accessed information through health professionals and...
- **50.5%** official websites of professional societies
- **77.6%** Females felt education about the menopause at school and...
- **64.3%** ...in doctors' surgeries would be best

A lot of females expressed how they wished their GPs were better informed, and many felt "dismissed and unsupported" by their GPs

References

1. Carter AE, Merriam S. Menopause. Med Clin North Am. 2023;107(2):199-212.
2. Davis S et al. Menopause. Nat Rev Dis Primers. 2015;1:15004
3. Nappi RE et al. Prevalence and quality-of-life burden of vasomotor symptoms associated with menopause: a European cross-sectional survey. Maturitas. 2023;167:66-74.
4. Aggarwal NR et al. Sex differences in ischemic heart disease: advances, obstacles, and next steps. Circ Cardiovasc Qual Outcomes. 2018;11(2):e004437.
5. Yong EL, Logan S. Menopausal osteoporosis: screening, prevention and treatment. Singapore Med J. 2021;62(4):159-66.
6. Nappi RE et al. Global cross-sectional survey of women with vasomotor symptoms associated with menopause: prevalence and quality of life burden. Menopause. 2021;28(8):875-82.
7. House of Commons, Women and Equalities Committee. Menopause and the workplace survey results. 2022. Available at: https://committees.parliament.uk/publications/8995/documents/152634/default/. Last accessed: 16 May 2023.
8. Ipsos. Global views on menopause: a global advisor survey. 2022. Available at: https://www.ipsos.com/sites/default/files/ct/news/documents/2022-10/Ipsos-Global%20Views%20on%20Menopause.pdf. Last accessed: 16 May 2023

Key

AMH: anti-Müllerian hormone; CVD: cardiovascular disease; FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone; GP: general practitioner; LH: luteinising hormone; HCP: healthcare professional; VMS: vasomotor symptoms.

EMJ

The Effects of Endocrine Disrupting Chemicals on the Outcomes of Pregnancy and Fertility Treatments Should Not Be Underestimated

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Infertility rates are rising. Infertility is generally defined as the failure to establish clinical pregnancy after 12 months of regular, unprotected sexual intercourse. Around the world, approximately one in six couples hoping to conceive are being diagnosed as infertile.[1] There are numerous well-established causes of infertility, including advanced age, polycystic ovary syndrome, and sexually transmitted infections. However, in more than 10% of couples the cause of infertility is unknown,[2] and the prevalence of this unexplained subfertility is increasing.[3] In parallel to this, the production of synthetic chemicals is increasing at dramatic rates across the globe, leading experts to question their effects on human health, including reproductive health. It is time to examine the evidence regarding endocrine disrupting chemicals (EDC) as a possible cause of infertility and subfertility in both males and females, and the detrimental impact they might have on consequential fertility treatments.

EDCs are exogenous chemicals with the ability to interrupt normal endocrine function in humans. They are used extensively throughout manufacturing, industrial, and agricultural sectors; however, most of us are exposed to them on a day-to-day basis through plastics, pharmaceuticals, cosmetics, and food preservatives. EDCs are pervasive chemicals, able to bio-accumulate within adipose tissue once ingested or inhaled. Their long-half lives and widespread use mean it is sadly impossible to escape these ubiquitous chemicals.

It has already been established that environmental pollution negatively impacts the fertility of all mammalian species,[4] and exposure to air pollutants, including carbon monoxide, is already associated with an increased risk of both miscarriage and stillbirth.[5] It is therefore simple to predict that EDCs might have a similar impact. Indeed, increasingly substantial evidence has shown negative associations between increasing EDC exposure and male and female fertility, chromosomal abnormalities, development and implantation problems within the embryo, and early pregnancy loss.[6]

The pathophysiological mechanisms behind these unwanted effects are poorly understood but are hypothesised to relate to the ability of some EDCs, such as bisphenol A (BPA) and triclosan, to competitively bind to hormone receptors and interfere with oestrogen signalling pathways.[6] BPA is also thought to inhibit aromatase activity, thereby inhibiting oestrogen synthesis and disrupting ovarian folliculogenesis and implantation.[7] The impact of this should not be underestimated. The presence of BPA in everyday products, including plastics, the lining of food and drink containers, as well as medical products, means that the general population are exposed to its effects on a daily basis.

Maternal exposure to EDCs is also an identifiable risk factor for several pregnancy complications, including recurrent miscarriage, hypertensive disorders such as pre-eclampsia, and pre-term birth.[8] A recent systematic review has examined convincing evidence to show that BPA exposure leads to foetal growth restriction and reduced birth weight, particularly when exposure occurs in the first 20 weeks of gestation.[9] Furthermore, other EDCs, including phthalates and perfluorooctane sulphonate, have been associated with a significantly increased chance of premature delivery.[10,11] These effects likely occur due to their accumulation within, or actions upon, the placenta, causing misregulation of trophoblastic signalling pathways and therefore altered cell viability.[8] These complications pose a significant risk to the health of both mother and child in the short and long term. Given that birth weight is such a significant predictor for future health outcomes, including obesity, diabetes, and cardiovascular disease, the downstream effects of EDC exposure are larger than we know.

Sadly, the patients who will feel the greatest impact from EDC exposure are those who are already more likely to have trouble conceiving and need to seek fertility treatment: older females. These females not only suffer from decreased ovarian reserve, as the number of oocytes is fixed from birth, with no way to replace them, but will have had longer exposure to the effects of various EDCs during their lifetime, which may well confound the effects of better-established risk factors, such as smoking, alcohol consumption, and air pollution, further reducing the chance of a successful pregnancy.

It is not just female fertility that is impacted by EDCs. Studies have shown male exposure to EDCs can impact normal testicular morphology and function. BPA has been associated with decreased sperm quality,[12] possibly via its oestrogenic and anti-androgenic effects,[13] whilst phthalate exposure is linked to hypospadias, cryptorchidism, and the development of testicular cancer.[14]

Unfortunately, EDCs can impact not just natural conception, but may also adversely influence the outcomes of fertility treatment. Several studies have shown relationships between increased exposure to BPA in females and poor outcomes of *in vitro* fertilisation (IVF) treatment. Specifically, serum BPA levels have been negatively associated with oocyte retrieval, oocyte maturation, fertilisation rates, and embryo quality.[15]

However, studying the impact of EDCs on fertility and IVF outcomes is hard to do. Human fertility is dependent on multiple factors, all underpinned by complex underlying processes such as folliculogenesis and spermatogenesis. One of the biggest challenges when studying their effects is that these chemicals are vast in number and have varying biochemical effects. The impact of one cannot, and should not, be generalised to be the impact of them all. Their ubiquitous nature further complicates matters, as they are nigh on impossible to avoid, and patients will be exposed to many different compounds at any one time. The presence of multiple different chemicals within the human body may therefore impact the effects seen by an individual chemical.

Research into how we can mitigate the harmful effects of EDCs is limited but ongoing. The most promising method appears to be via increased antioxidant intake. Oxidative stress has been shown to indicate BPA toxicity, and various antioxidants, including vitamins, herbs, and melatonin, may be useful to counteract its effects.[16] Other studies have shown a similar protective role, with vitamins C and E minimising the impact of phthalates and polychlorinated biphenyls.[17]

It is clear that EDCs pose significant risk to human reproductive health, with potential downstream effects lasting for years to come. Significant evidence has shown associations

between increasing EDC concentration, diminishing ovarian reserve, and poor fertility and IVF outcomes. The literature is occasionally conflicting, usually due to small sample sizes and a lack of control of possible confounding variables. However, the association between exposure to EDCs and poor reproductive outcomes is undeniable, and will have a substantial impact on public health. Further research is needed to examine the impact of EDCs in combination, and to understand their mechanisms of action, as identifying the risk of a single chemical is meaningless, given we are exposed to hundreds of these chemicals in combination from conception. Furthermore, research should also focus on how we can reduce their effects, possibly via a diet rich in antioxidants, or through alternative methods. In the meantime, a global strategy is urgently needed to prevent exposure to these chemicals, especially in females of child-bearing age, but ideally from birth, to mitigate their impact on the outcomes of pregnancy. Healthcare workers within reproductive medicine should be educated about the impacts of EDCs, and how to support patients to minimise their exposure and moderate their effects. At a population level, awareness campaigns should advise on the use of consumer products, particularly personal care products, and healthy diets, to avoid EDCs where possible. These methods will not only improve public health, but ensure as many parents as possible achieve the ultimate goal of reproduction: a healthy and happy baby.

References

1. Ravitsky V, Kimmins S. The forgotten men: rising rates of male infertility urgently require new approaches for its prevention, diagnosis and treatment. Biol Reprod. 2019;101(5):872-4.
2. Altmäe S et al. Allelic estrogen receptor 1 (ESR1) gene variants predict the outcome of ovarian stimulation in in vitro fertilization. Mol Hum Reprod. 2007;13(8):521-6.
3. Li Q et al. Association between exposure to airborne particulate matter less than 2.5 μm and human fecundity in China. Environ Int. 2021;146:106231.
4. Borght MV, Wyns C. Fertility and infertility: definition and epidemiology. Clin Biochem. 2018;62:2-10.
5. Grippo A et al. Air pollution exposure during pregnancy and spontaneous abortion and stillbirth. Rev Environ Health. 2018;33(3):247-64.
6. Green MP et al. Endocrine disrupting chemicals: impacts on human fertility and fecundity during the peri-conception period. Environ Res. 2021;194:110694.
7. Karwacka A et al. Exposure to modern, widespread environmental endocrine disrupting chemicals and their effect on the reproductive potential of women: an overview of current epidemiological evidence. Hum Fertil (Camb). 2019;22(1):2-25.
8. Yang C et al. A mechanism for the effect of endocrine disrupting chemicals on placentation. Chemosphere. 2019;231:326-36.
9. Vrachnis N et al. A systematic review of bisphenol A from dietary and non-dietary sources during pregnancy and its possible connection with fetal growth restriction: investigating its potential effects and the window of fetal vulnerability. Nutrients. 2021;13(7):2426.
10. Ferguson KK et al. Environmental phthalate exposure and preterm birth. JAMA Pediatr. 2014;168(1):61-7.
11. Deji Z et al. Association between maternal exposure to perfluoroalkyl and polyfluoroalkyl substances and risks of adverse pregnancy outcomes: a systematic review and meta-analysis. Sci Total Environ. 2021;783:146984.
12. Radwan M et al. Urinary bisphenol a levels and male fertility. Am J Mens Health. 2018;12(6):2144-51.
13. Wetherill YB et al. In vitro molecular mechanisms of bisphenol A action. Reprod Toxicol. 2007;24(2):178-98.
14. Nordkap L et al. Regional differences and temporal trends in male reproductive health disorders: semen quality may be a sensitive marker of environmental exposures. Mol Cell Endocrinol. 2012;355:221-30.
15. Ziv-Gal A, Flaws JA. Evidence for bisphenol A-induced female infertility: a review (2007-2016). Fertil Steril. 2016;106(4):827-56.
16. Meli R et al. Oxidative stress and BPA toxicity: an antioxidant approach for male and female reproductive dysfunction. Antioxidants. 2020; 9(5):405.
17. Baldi F, Mantovani A. A new database for food safety: EDID (Endocrine disrupting chemicals - Diet Interaction Database). Ann Ist Super Sanita. 2008;44(1):57-63.

Vertical Transmission in Pregnancy with COVID-19 For the January–April 2021 Period at the Prof. dr. I.G.N.G. Ngoerah General Hospital, Denpasar, Indonesia

Editor's Pick

Females who are pregnant are more susceptible to various diseases due to their immunocompromised state; however, the vertical transmission of SARS-CoV-2 is yet to be formally defined. Thus, Pranamartha et al. aim to describe the incidence of COVID-19, and the vertical transmission of COVID-19 in pregnancies, at the Prof. Dr. I.G.N.G. Ngoerah Denpasar General Hospital, Bali, Indonesia, from January–April 2021. The cross-sectional observational study identified 15 females who tested positive for COVID-19, resulting in one baby with reactive examination results, and suggesting the risk of vertical transmission was increased when compared to a pregnancy without COVID-19 infection. Overall, the research team report some interesting preliminary results, which warrant further investigation.

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Abstract

Background: COVID-19 is an infectious disease that can infect anyone, including pregnant females, a population that is susceptible to various infections. This has become a challenge because of the risk of vertical transmission and increased foeto-maternal mortality. That authors' purpose was to present the

incidence of pregnancy with COVID-19 and the vertical transmission in pregnancies with COVID-19.

Methods: This study used cross-sectional observational research and was carried out at the Prof. dr. I.G.N.G. Ngoerah Denpasar General Hospital, Denpasar, Indonesia, from January–April 2021. The authors used primary data from pregnant females who presented at the hospital with positive severe acute respiratory syndrome coronavirus 2 PCR results. Additional instruments included data collection forms and medical records.

Results: Based on primary data, a total of 15 pregnant females with COVID-19 were identified from a total of 165 deliveries in that period. The prevalence of COVID-19 events in pregnant females was 9.09%. It was found that the rapid blood antibody results for all infants had non-reactive results for IgM. It can be assessed that the relative risk of transmitting COVID-19 antibodies from mother to foetus is three times (risk ratio: 3.00; 95% confidence interval: 1.56–64.26). One baby was found with reactive examination results so that the prevalence ratio obtained was 11.7 (prevalence ratio: 11.7; 95% confidence interval: 1.63–35.57).

Conclusion: The prevalence rate of pregnant females with COVID-19 at the Prof. dr. I.G.N.G. Ngoerah General Hospital for the period of January–April 2021 was 9.09%. COVID-19 infection in pregnancy can increase the risk of vertical transmission of COVID-19 by 11.7 times compared with pregnancy without COVID-19 infection.

Key Points

1. Despite a decrease in COVID-19 infection rates, infection during pregnancy may result in poor outcomes for some, with a mortality rate of 1.6%, and potential vertical transmission.

2. In this study, the authors describe the incidence of pregnancy with COVID-19, along with the vertical transmission of COVID-19, in pregnancies at the Prof. dr. I.G.N.G. Ngoerah Denpasar General Hospital, Denpasar, Indonesia, from January–April 2021.

3. Results indicate that females with COVID-19 who are pregnant have an 11.7 times higher risk of vertically transmitting it to their babies compared with those without COVID-19 infection.

INTRODUCTION

COVID-19 is an infectious disease that has become a worldwide pandemic. This disease can infect anyone who has been exposed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), including pregnant females, who are a population that is more susceptible to various infections due to their immunocompromised state.[1,2] Here in lies the challenge. Since there would be the possibility of a different COVID-19 clinical course in pregnant females, with different treatment considerations to the risk of vertical transmission and increased foeto-maternal mortality, studies regarding COVID-19 in pregnant females need to be developed further to ensure the best care possible for this specific population group.[3-5]

Indonesia has been one of the biggest hotspots for COVID-19, especially in Southeast Asia. As of 15th March 2023, there have been well over 6 million confirmed cases since 2020, and over 160,000 deaths.[6] Although epidemiological research on COVID-19 in pregnancy in Indonesia is still quite limited, according to the Routine Family Health Data from the Indonesian Ministry of Health, there has been an increase in the number of maternal deaths during the pandemic in areas with a distribution of COVID-19 cases.[7,8]

Physiological changes in the immune system of pregnant females have long been known to increase susceptibility to infections in general. Apart from that, the virus is also thought to be able to bind to angiotensin converting enzyme (ACE) 2 receptors in various body

tissues, including the placenta, hence allowing it to cross the placental barrier and be transmitted vertically.[9]

Vertical transmission of SARS-CoV-2 is yet to be formally defined, but Blumberg et al.[10] proposed that vertical transmission is detected if the mother is positive for COVID-19 between 14 days before birth and up to 2 days after birth; the virus is detected at the neonate's respiratory tract or blood sample, amniotic fluid, or umbilical cord blood; and there are signs of persistence from either a positive swab of the respiratory tract after 24 hours of life, or a positive SARS-CoV-2 IgM in the first 7 days of life. Mahyuddin et al.[11] also stated that to diagnose transplacental infection, samples taken from the placenta, umbilical cord, neonatal airway, rectum, or amniotic fluid (prior to rupture of membranes) for real-time PCR are needed.

Even though incidence rates of COVID-19 have been decreasing, the authors still consider the fact that COVID-19 infection in pregnancy may result to poor outcomes in some, with a mortality rate of 1.6%, and that it may cause vertical transmission.[12-15] Therefore, the authors are interested in further research to provide knowledge about the epidemiology, characteristics, and impacts of COVID-19 infection in pregnancy. In this study, the authors will describe the incidence of pregnancy with COVID-19, along with the vertical transmission of COVID-19 in pregnancies at the Prof. dr. I.G.N.G. Ngoerah Denpasar General Hospital, Denpasar, Indonesia, from January–April 2021.

METHODS

This study used a cross-sectional, observational research method and was carried out at the Central General Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar from January–April 2021. The reachable population included pregnant females with COVID-19 at this hospital during this period. The inclusion criteria for eligibility were: all obstetrics patients with confirmed COVID-19 from reverse transcription-PCR (RT-PCR) who gave birth at General Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar from January–April 2021, who have signed the informed consent; mothers who went through Caesarean section delivery; and all babies born from the confirmed COVID-19 mothers. The participants were excluded if the obstetric patient who was about to give birth had a negative RT-PCR result, they had incomplete data, or the mother gave birth vaginally.

The primary data for this study would be the pregnant females who came to Prof. Dr. I.G.N.G. Ngoerah referred from primary or secondary healthcare facilities that brought positive SARS-CoV-2 RT-PCR results. Additional instruments in this study were data collection forms and medical records. The data of this research were obtained from pregnant females with COVID-19 who were about to give birth and patients in the COVID-19 isolation room that were selected according to the research sample criteria, namely patients who were pregnant during the study period.

After the pregnancy of the females who were COVID-19-positive were terminated by Caesarean section, blood samples to test for IgM and IgG anti-SARS-CoV-2 were taken from the newborns to indicate the vertical transmission of COVID-19.

The data taken was entered into the Microsoft Excel computer software (Microsoft, Redmond, Washington, USA). The data that was obtained was then tabulated and analysed to calculate the prevalence of pregnancy with COVID-19 and the prevalence of the vertical transmission ratio of COVID-19.

RESULTS

Based on the primary data of pregnant females with COVID-19 at the Central General Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar from January–April 2021, a total of 15 pregnant females were positive with COVID-19 from a total of 165 deliveries in that period, so that the prevalence of COVID-19 infection in pregnant females at this hospital for that period was 9.09%. An overview of the prevalence of pregnant females with COVID-19 infection can be seen in Table 1.

Table 1: Prevalence of pregnancy with COVID-19 infection at the Prof. dr. I.G.N.G. Ngoerah General Hospital, Denpasar, Indonesia.

			Prevalence
SARS-CoV-2 RT-PCR	Positive	15	9.09%
	Negative	150	
	Total	165	

The serum IgM and IgG findings from the 15 neonates born to mothers with COVID-19 only resulted in one positive result, which was positive only for IgG, but not IgM. After calculating the prevalence ratio by comparing the positive group with the negative group, it is found that the PR is 11.7% (PR: 11.7; 95% CI: 1.63–35.57).

CI: confidence interval; PR: prevalence ratio; RT-PCR: reverse transcription PCR; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

DISCUSSION

Epidemiological data on maternal cases of COVID-19 are still minimal, but the prevalence from this study is consistent with several studies, which state that the prevalence of COVID-19 in pregnancy ranges from 3–20%.[16]

In this study, it was found that the rapid blood antibody results for all newborns were non-reactive for IgM. This is because IgM is usually unable to be transferred from mother to foetus due to its large macromolecular structure that is incapable of crossing the intact placental blood barrier.[17] These results are different from one study conducted in Wuhan, China, which found that there were newborns born to mothers with COVID-19 who had positive IgM anti-SARS-CoV-2 at birth.[18] This reactive IgM result can occur if the virus itself, rather than the IgM antibody, is transmitted through the placenta, so that the baby will produce their own IgM after having been infected.[17,19]

Since it has been known that SARS-CoV-2 binds to ACE2 receptors, and there is an increased ACE2 in pregnant females, with a limited number on the placenta, vertical infection through the placenta still may occur.[20,21] Until now, there are still researchers who doubt that SARS-CoV-2 can be transmitted vertically; instead they propose that the reactive IgM findings in newborns might be caused by a damaged placenta, which allows the mother's IgM to be transmitted to the foetus.[22]

Damages occurring to the placenta could also be explained by the infection of SARS-CoV-2, as a variety of histopathological changes were observed in COVID-19 positive pregnancies, such as fibrin and thrombus formation, avascular villi, and other decidual arteriopathies. The findings of placental pathology cause malperfusion observed from both the maternal and foetal side and could be associated with the worse maternal and foetal outcomes in pregnancy with COVID-19.[23-26] One case report conducted by Hosier et al.[27] described that high viral load of SARS-CoV-2 RNA resides mostly in the syncytiotrophloblast cells of the placenta. The abundance of SARS-CoV-2 in the syncytiotrophloblast cells might be explained by how ACE2 genes are expressed in those cells at 6–14 weeks of gestation, including in the perivascular cells in decidua, and villous cytotrophoblast.[28]

It is suspected that there may be a possibility of vertical transmission due to studies showing the discovery of ACE2 receptors even in small amounts in the placenta, which allows vertical transmission through the placenta.[21] When

there is a bond between SARS-CoV-2 and the ACE2 receptor, transmembrane protease serine enzyme 2 will be activated and facilitate the virus to pass through cells.[29] This enzyme is known to be expressed in villous cytotrophoblasts, epithelial glandular cells, and also expressed minimally in syncytiotrophoblast cells.[28] This makes it possible to find the SARS-CoV-2 virus RNA in the placenta or amniotic membranes as reported by Penfield et al.[30]

This study showed similar results with one case report from Wuhan of a neonate born from a female who was IgG anti-SARS-CoV-2 positive, who had normal IgM levels but strong positive IgG results.[31]

Another study conducted on six neonates from the same region showed strong positive IgG, but all had negative IgM, except for one. Out of the six newborns, one of them had a positive IgG value up to 150 days, and two stayed positive up until the 180th day. The mothers of the two infants also still showed positive IgG on the 180th day, indicating that maternal IgG levels could possibly provide protective IgG against COVID-19 for their infants.[32] The amount of IgG antibody titre found in the infants depends on the amount of IgG titre of the mother. Pregnant females who have been infected with SARS-CoV-2 for more than 2 weeks will provide a more adequate antibody titre in the infant.[33] Even so, the persistent IgG values on the infants still could be affected by other external factors such as breastfeeding. Breastmilk has been proven to carry anti-SARS-CoV-2 IgG antibodies, whether the antibodies are created from previous COVID-19 vaccination or infection. The IgG antibody is also evident from infants' stool.[34]

It has also already been proven that vaccination of mothers against SARS-CoV-2 during pregnancy results to antispike IgG being formed in their infants for up to 3 months.[35] Regardless, positive IgG findings in infants are known to help neutralise SARS-CoV-2.[36]

The presence of IgG against SARS-CoV-2 in newborns who are negative on RT-PCR examination and born to mothers with COVID-19 indicates the possibility of transmission of antibodies from mother to child.[37] Maternal immunity can cross the blood–placenta barrier, which can cause the formation of passive immunity in the foetus.[38] Therefore, from this study it can be assessed that the relative risk of transmitting COVID-19 antibodies from mother to foetus is 3 times (risk ratio: 3.00; 95% confidence interval [CI]: 1.56–64.26). In contrast to IgM, IgG can be passively transferred from mother to foetus across the blood–placental barrier. Transfer of IgG usually begins at the end of the second trimester and reaches high levels at birth.[17,18] Even though the presence of IgG in the infant's body indicates that the baby has passive immunity, the duration of passive immunity from the mother's IgG is still unclear.[18] There are studies that state that this protective effect of passive immunity does not last long and will disappear after 2 months.[33]

The possibility of negative IgG findings in the other newborns of this study could be explained due to low maternal IgG titres, or the deliveries happening before IgG transfer to the foetus could occur.[39] It should also be put into consideration that serum IgM and IgG can be detected within days up to 1–3 weeks after COVID-19 infection,

Table 2: Features of vertical transmission in pregnancy with COVID-19 infection.

		Maternal COVID-19		PR (%)	95% CI
		Positive	Negative		
Neonate serology	Positive	1	0	1.7	10.63–35.57
	Negative	14	150		

CI: confidence interval; PR prevalence ratio.

and IgM antibodies decay earlier than IgG, hence the timing from maternal COVID-19 infection to taking the blood serum sample might affect the subsequent findings.[40,41]

From the results of this study, there were 165 pregnant females from January–April 2021. Out of 165 pregnant females there were 15 cases positive for COVID-19. From these data, one baby was found with reactive examination results so that the prevalence ratio (PR) could be calculated by comparing the number of cases in the test group with the control group and the results obtained were 11.7 (PR: 11.7; 95% CI: 1.63–35.57 [Table 2]). PR results >1 indicate that pregnant females with COVID-19 have a risk of vertically transmitting it to their babies 11.7 times higher compared with those without COVID-19 infection. Nonetheless, until now the evidence of vertical transmission of COVID-19 is still being investigated.

A case study from Iran reported positive SARS-CoV-2 RNA results in the amniotic fluid of a premature baby, who then showed positive nasopharyngeal swab RT-PCR results 24 hours post-partum, but negative RT-PCR results from samples taken from the vaginal secretion and umbilical cord blood.[37]

Another case study conducted by Kirtsman et al.[42] reported the possibility of congenital infection with SARS-CoV-2 as evident by positive RT-PCR swab results from samples taken from the newborn's nasopharynx, blood (Day 4), stool (Day 7), the placenta, and the mother's breast milk, along with maternal vaginal swabs. In a larger study of 666 newborns of females positive for SARS-CoV-2, 28 infants (4%) were infected with SARS-CoV-2 after birth.[43] Meta-analysis research conducted by Kotlyar et al.[44] showed that the combined proportion of vertical transmission of SARS-CoV-2 from 936 neonates born from mothers with COVID-19 was 3.2% (95% CI: 2.2–4.3). Other sites tested for SARS-CoV-2 in this study yielded a variety of positive results, such as 0.0% in amniotic fluid and urine samples, 7.7% in placental samples, 3.6% from the cord blood, 9.7% in anal or rectal swabs, and a seropositivity of 3.7% of neonates. Another study from China showed that the risk of vertical transmission is 16 out of 1,000 live births.[45] This shows that the risk of vertical transmission is considerably small.

Some other literature reports that there are no documented cases of intrauterine transmission, and there are cases that only find an increase in the level of COVID-19 IgM in neonates aged 1–2 days. A positive IgM result is not definitive evidence of intrauterine infection because of the possibility of false positives and cross-reactivity that occurs. Maternal immunity can cross the placental blood barrier, which can lead to the formation of passive immunity in the foetus. Moreover, in many cases early infant infection may have occurred due to postnatal contact with infected parents or caregivers.[46-49]

The method of delivery has also been a topic of concern regarding the risk of vertical transmission. Some studies have found that Caesarean section delivery is more commonly done for females infected with COVID-19.[50,51] Vertical transmission may occur through the placenta, through direct contact with maternal blood or vaginal secretion during birth, and through breastfeeding.[51] Some may argue that vaginal delivery poses a higher risk of vertical transmission, since there would be a direct contact between the newborn and cervicovaginal secretion, but instead a meta-analysis study from Indonesia, conducted by Sarastry et al.,[51] showed that vaginal delivery does not hold a greater risk of vertical transmission of SARS-CoV-2 to newborns compared with Caesarean section deliveries, hence the method of delivery should be based on the obstetric indications. Their findings are in line with the results from the study conducted by Lopian et al.[52] and Cai et al.[53]

Even though vertical transmission is yet to be proven, severity of maternal COVID-19 infection could also be one of the determining factors of vertical transmission happening, since worse COVID-19 severity causes an increase in serum IgM and IgG antibody production against SARS-CoV-2.[54]

Measures should also be taken to prevent vertical and post-partum transmission of SARS-CoV-2 from mothers to their newborns. One cohort study completed by Tavakoli et al.[55] found that using remdesivir for the treatment protocol of pregnant females with COVID-19 is shown to reduce vertical transmission and reduce neonatal intensive care unit admissions. It is also not a contraindication for infants to receive breastmilk from their COVID-19-positive mothers, although

its safety and preventive measures against SARS-CoV-2 transmission should be noted, considering that 70% of neonate infections occur through postpartum transmission. Therefore, practising hand hygiene, wearing face masks, and cleaning breast pumps properly before and after breast milk expression is crucial to prevent further transmission.[56]

One of the limitations of this study was using serum IgM and IgG to describe vertical transmission of SARS-CoV-2 when some studies have shown that anti-SARS-CoV-2 IgG can be transmitted to the foetus post-COVID-19 vaccination; hence, COVID-19 vaccination status should be recorded and considered for future studies.[57-59] Moreover, antibody serology testing is less sensitive and specific for COVID-19 detection; therefore, using RT-PCR for diagnosing COVID-19 is preferred and is considered to be more representative for vertical transmission.[60] The number of COVID-19 in pregnant cases presented in this study is still very limited, so results of this study should be interpreted according to context. Further research with a longer study design might solve the problem of the amount number of samples included.

Certain maternal and neonate variables, such as severity of COVID-19 and gestational age, were also not taken into consideration. The sample characteristics should be considered in future studies to anticipate their effects on the study outcomes.

CONCLUSION

To sum up the findings of this study, the incidence of pregnant females with COVID-19 at the Central General Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar from January–April 2021 was 15 cases out of 165 caesarean section deliveries, with a prevalence rate of 9.09%.

According to this study, COVID-19 infection in pregnancy can increase the risk of vertical transmission of COVID-19 by 11.7 times compared with pregnancy without COVID-19 infection. Further studies considering the duration of study design, amount of sample included, as well as other variables such as vaccination history, COVID-19 severity, and gestational age should be taken into consideration to create a more reliable study.

References

1. World Health Organization (WHO). Report of the WHO-China joint mission on coronavirus disease 2019 (COVID-19). 2020. Available at: https://www.who.int/publications/i/item/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19). Last accessed: 30 July 2021.
2. World Health Organization (WHO). WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. 2020. Available at: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020. Last accessed: 30 July 2021.
3. Task Force Handling Covid-19. Analysis of Indonesian COVID-19 data. 2020. Available at: https://covid19.go.id/artikel/2020/09/01/analisis-data-covid-19-indonesia-update-30-agustus-2020. Last accessed: 30 July 2021.
4. Han Y, Yang H. The transmission and diagnosis of 2019 novel coronavirus infection disease (COVID-19): a Chinese perspective. J Med Virol. 2020;92(6):639-44.
5. Bai Y et al. Presumed asymptomatic carrier transmission of COVID-19. JAMA. 2020;323(14):1406-7.
6. World Health Organization (WHO). WHO coronavirus disease (COVID-19) dashboard. Available from: https://covid19.who.int. Last accessed: 16 March 2023.
7. Indonesian Ministry of Health. Handbook for prevention and control of coronavirus disease (COVID-19). 2020. Available at: https://infeksiemerging.kemkes.go.id/download/REV-04_Pedoman_P2_COVID-19__27_Maret2020_TTD1.pdf. Last accessed: 30 July 2021.
8. Ministry of Health Republic of Indonesia. Guidelines for services for pregnant females, childbirth, postpartum, and newborns. Guidelines for services for pregnant, maternity, postpartum and newborn mothers. 2019. Available at: https://apps.who.int/iris/handle/10665/249580. Last accessed: 30 July 2021.
9. Kolifarhood G et al. Epidemiological and clinical aspects of COVID-19; a narrative review. Arch Acad Emerg Med. 2020;8(1):e41.
10. Blumberg DA et al. Vertical transmission of SARS-CoV-2: what is the optimal definition? Am J Perinatol. 2020;37(8):769-72.
11. Mahyuddin AP et al. Mechanisms and evidence of vertical transmission of infections in pregnancy including SARS-CoV-2s. Prenat Diagn. 2020;40(13):1655-70.
12. Working group PP POGI Reproductive Tract Infection. Recommendations for handling corona virus (COVID-19) in maternal (pregnancy, maternity and postpartum) revision 2. 2020. Available at: https://pogi.or.id/publish/wp-content/uploads/2021/06/Revisi-Rekomendasi-POGI-utk-Bumil-dengan-Covid-19-.pdf. Last accessed: 30 July 2021.
13. Vouga M et al. Maternal outcomes and risk factors for COVID-19 severity among pregnant females. Sci Rep. 2021;11(1):13898.

14. Allotey J et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. BMJ. 2020;370:m3320.
15. Gorbalenya AE et al. Severe acute respiratory syndrome-related coronavirus: the species and its viruses–a statement of the coronavirus study group. Nat Microbiol. 2020;5:536-44.
16. Jamieson DJ, Rasmussen SA. An update on COVID-19 and pregnancy. Am J Obstet Gynecol. 2022;226(2):177-86.
17. Zeng H et al. Antibodies in infants born to mothers with COVID-19 pneumonia. JAMA. 2020;323(18):1848-9.
18. Gao J, et al. Disappearance of SARS-CoV-2 antibodies in infants born to females with COVID-19, Wuhan, China. Emerg Infect Dis. 2020;26(10):2491-4.
19. Li X et al. Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal. 2020;10(2):102-108.
20. Christyani F, Padang A. Vertical transmission of COVID 19 during pregnancy. Cermin Dunia Kedokteran. 2020;47(9):663-7.
21. Hosier H et al. SARS-CoV-2 infection of the placenta. J Clin Invest. 2020;130(9):4947-53.
22. Cavaliere A et al. Passive immunity in newborns from SARS-CoV-2-infected mother. J Med Virol. 2021;93(3):1810-3.
23. Shanes ED et al. Placental pathology in COVID-19. Am J Clin Pathol. 2020;154(1):23-32.
24. Giordano G et al. COVID-19 in pregnancy: placental pathological patterns and effect on perinatal outcome in five cases. Diagn Pathol. 2021;16(1):88.
25. Al-Rawaf SA et al. Correlation between pregnancy outcome and placental pathology in COVID-19 pregnant females. Infect Dis Obstet Gynecol. 2022;2022:8061112.
26. Pacu I et al. SARS-CoV-2 Infection during pregnancy and histological alterations in the placenta. Diagnostics (Basel). 2022;12(9):2258.
27. Hosier H et al. SARS-CoV-2 infection of the placenta. J Clin Invest. 2020;130(9):4947-53.
28. Dhaundiyal A et al. Is highly expressed ACE 2 in pregnant females "a curse" in times of COVID-19 pandemic? Life Sci. 2021;264:118676.
29. Deniz M, Tezer H. Vertical transmission of SARS CoV-2: a systematic review. J Matern Fetal Neonatal Med. 2020;35(14): 2655-62.
30. Penfield C et al. Intrauterine vertical transmissibility of SARS-CoV-2: the evidence is evolving. Am J Obstet Gynecol MFM. 2020;2(4):100227.
31. Gao W et al. A newborn with normal IgM and elevated IgG antibodies born to an asymptomatic infection mother with COVID-19. Aging (Albany NY). 2020;12(17):16672-4.
32. Mo H et al. Detectable antibodies against SARS-CoV-2 in newborns from mothers infected with COVID-19 at different gestational ages. Pediatr Neonatol. 2021;62(3):321-3.
33. Wang X et al. Dynamic changes of acquired maternal SARS-CoV-2 IgG in infants. Sci Rep. 2021;11(1):8021.
34. Pace RM et al. COVID-19 and human milk: SARS-CoV-2, antibodies, and neutralizing capacity. medRxiv. 2020;2020.09.16.20196071.
35. Golan N et al. Quantitative analysis of vertical transmission of maternal SARS-CoV-2 antibodies to neonates and young infants following immunization during pregnancy. J Pediatric Infect Dis Soc. 2022;11(11):506-9.
36. Stafford LS et al. Detection of SARS-CoV-2 IgA and IgG in human milk and breastfeeding infant stool 6 months after maternal COVID-19 vaccination. J Perinatol. 2023;DOI:10.1038/s41372-022-01581-5.
37. Zamaniyan M et al. Preterm delivery, maternal death, and vertical transmission in a pregnant woman with COVID-19 infections. Prenate Diagn. 2020;40(13): 1759-61.
38. Liu Y et al. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Travel Med. 2020;27(2):taaa021.
39. Robinson T. Antibody testing of infants born to asymptomatic COVID-19 positive mothers. Neonatology Today. 2021;16(10):3-7.
40. Centers for Disease Control and Prevention (CDC). Interim guidelines for COVID-19 antibody testing. 2022. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing/antibody-tests-guidelines.html. Last accessed: 11 December 2021.
41. Guo L et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis. 2020;71(15):778-85.
42. Kirtsman M et al. Probable congenital SARS-CoV-2 infection in a neonate born to a woman with active SARS-CoV-2 infection. CMAJ. 2020;192(24):E647-50.
43. Walker KF et al. Maternal transmission of SARS-COV-2 to the neonate, and possible routes for such transmission: a systematic review and critical analysis. BJOG. 2020;27(11):1324-36.
44. Kotlyar A et al. Vertical transmission of coronavirus disease 2019: a systematic review and meta-analysis. Am J Obstet Gynecol. 2021;224(1):35-53.
45. Goh XL et al. Incidence of SARS-CoV-2 vertical transmission: a meta-analysis. Arch Dis Child Fetal Neonatal Ed. 2021;106(1):112-113.
46. Qiancheng X et al. Coronavirus disease 2019 in pregnancy. Int J Infect Dis. 2020;95:376-383.
47. Schwartz DA. An analysis of 38 pregnant females with COVID-19, their newborn infants, and maternal-fetal transmission of SARS-CoV-2: maternal coronavirus infections and pregnancy outcomes. Arch Pathol Lab Med. 2020;144(7):799-805.
48. Della Gatta AN et al. Coronavirus disease 2019 during pregnancy: a systematic review of reported cases. Am J Obstet Gynecol. 2020;223(1):36-41.
49. Kimberlin D, Stagnos S. Can SARS-CoV-2 infection be acquired in utero?: more definitive evidence is needed. JAMA. 2020;323(18):1788-9.
50. Debrabandere ML et al. A review on mode of delivery during COVID-19 between December 2019 and April 2020. Am J Perinatol. 2021;38(4):332-41.
51. Sarastry R et al. Delivery routes in pregnancy with COVID-19 and the risk of intrapartum vertical transmission: a meta-analysis. Med J Indones. 2021;30(2):116-22.
52. Lopian M et al. Safety of vaginal delivery in females infected with COVID-19. Pediatr Neonatol. 2021;62(1):90-96.
53. Cai J et al. Cesarean section or vaginal delivery to prevent

possible vertical transmission from a pregnant mother confirmed with COVID-19 to a neonate: a systematic review. Front Med (Lausanne). 2021;8:634949.

54. Hu WT et al. Antibody profiles according to mild or severe SARS-CoV-2 infection, Atlanta, Georgia, USA, 2020. Emerg Infect Dis. 2020;26(12):2974-8.
55. Tavakoli N et al. Is it possible to reduce the rate of vertical transmission and improve perinatal outcomes by inclusion of remdesivir in treatment regimen of pregnant females with COVID-19? BMC Pregnancy Childbirth. 2023;23(1):110.
56. National Institute of Health (NIH). Special considerations during pregnancy and after delivery. 2023. Available at: https://files.covid19treatmentguidelines.nih.gov/guidelines/section/section_44.pdf. Last accessed: 8 May 2023.
57. Flannery DD et al. Comparison of maternal and neonatal antibody levels after COVID-19 vaccination vs SARS-CoV-2 infection. JAMA Network Open. 2022;5(11):e2240993.
58. Paul G, Chad R. Newborn antibodies to SARS-CoV-2 detected in cord blood after maternal vaccination – a case report. BMC Pediatr. 2021;21(1):138.
59. Shook LL et al. Durability of anti-spike antibodies in infants after maternal COVID-19 vaccination or natural infection. JAMA. 2022;327(11):1087-9.
60. Cascella M et al. Features, evaluation, and treatment of coronavirus (COVID-19) [Internet] (2022). Treasure Island: StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK554776/. Last accessed: 10 October 2022.

Ovarian Hyperstimulation Syndrome Post-dilation and Evacuation of a Hydatidiform Mole: A Case Report

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Abstract

The present study reports a complete hydatidiform mole presenting with ovarian hyperstimulation syndrome after dilatation and evacuation. A 30-year-old female came to the emergency room with abdominal pain and genital bleeding at 14 weeks of pregnancy. Ultrasonography revealed vesicular cystic swelling of chorionic villi with high human chorionic gonadotrophin (625,000 IU/L). Dilatation and evacuation was performed. Symptoms of ovarian hyperstimulation syndrome appeared on the 8th day after dilatation and evacuation, and bilateral enlarged multicystic ovaries were found, measuring 13.07×8.45×9.77 cm on the left and 9.02×8.45×5.5 cm on the right. The cysts gradually reduced in size and finally disappeared at Day 42, and human chorionic gonadotrophin reached below cut-off value at Day 72.

Key Points

1. Although ovarian hyperstimulation syndrome (OHSS) accompanied by natural pregnancy is rare, in case of hydatidiform mole there is cystic proliferation of chorionic villi and release of excess human chorionic gonadotrophins, by which ovaries are hyperstimulated.

2. This manuscript describes clinical assessment, specific laboratory parameters, treatment options, and positive consequence in OHSS after dilatation and evacuation of hydatidiform mole.

3. Spontaneous onset of OHSS can be a complication of hydatidiform mole and the condition worsens after dilatation and evacuation. Clinicians should be aware of ovarian cysts accompanied OHSS, as clinical assessment and timely intervention is helpful to avoid complications.

INTRODUCTION

Ovarian hyperstimulation syndrome (OHSS) is common following ovarian stimulation by clomifene citrate or follicle stimulating hormone.[1] This stimulation causes exposure of ovaries to human chorionic gonadotrophins (hCG) or leutinising hormone. As ovaries are hyperstimulated, there is increased production of pro-inflammatory mediators like vascular endothelial growth factors (VEGF). Due to increased endothelial permeability by VEGF, clinical manifestations occur, such as ascites, pleural effusion, haemoconcentrations (increased haematocrit >45%), and in severe cases thromboembolism.[2] OHSS is rare in naturally conceived pregnancy, but any type of molar pregnancy, complete or partial mole, can be complicated by OHSS. Usually this condition worsens after dilation and evacuation (D&E).[3]

CASE REPORT

A 30-year-old female, mother of 2 children (last child aged 5 years), with naturally conceived pregnancy, presented to the emergency room at 14 weeks of gestation with complaints of abdominal pain and genital bleeding for 6 hours. On admission, patient was mildly anaemic, with pulse rate of 90 beats/min, blood pressure of 110/65 mmHg, height of 154 cm, weight of 42 kg, and BMI of 17.71 kg/m². Uterine height was at the level of the umbilicus, which is larger than the corresponding gestational period of 14 weeks. Genital bleeding was present and ultrasonography revealed 'snow storm' appearance, suggestive of cystic swelling of chorionic villi, and absence of foetal parts. Laboratory parameters revealed white blood cells of 10,600 /µL, haematocrit of 40%, albumin of 4.3 mg/dL, and hCG of 625,000 IU/L (normal <5 IU/L). Considering all these parameters, including clinical features at 14 weeks gestation of abdominal pain and genital bleeding with increased fundal height; radiological findings of 'snow storm' appearance suggestive of cystic swelling of chorionic villi, and absence of foetal parts; and hCG laboratory value of 625,000 IU/L, the authors diagnosed this as a case of hydatidiform mole. D&E was subsequently performed.

After evacuation, the authors found a profuse amount of uterine contents, which were cystic without any foetal component. Histopathology revealed complete hydatidiform mole. After evacuation, hCG decreased to 22,803 IU/L and 12,803 IU/L at Day 2 and Day 4, respectively. It gradually reached cut-off level of less than 5 IU/L at Day 72 (here cut-off level is a value of hCG, where below this level is considered negative or not detected and above this value is positive, with a normal value <5 IU/L).

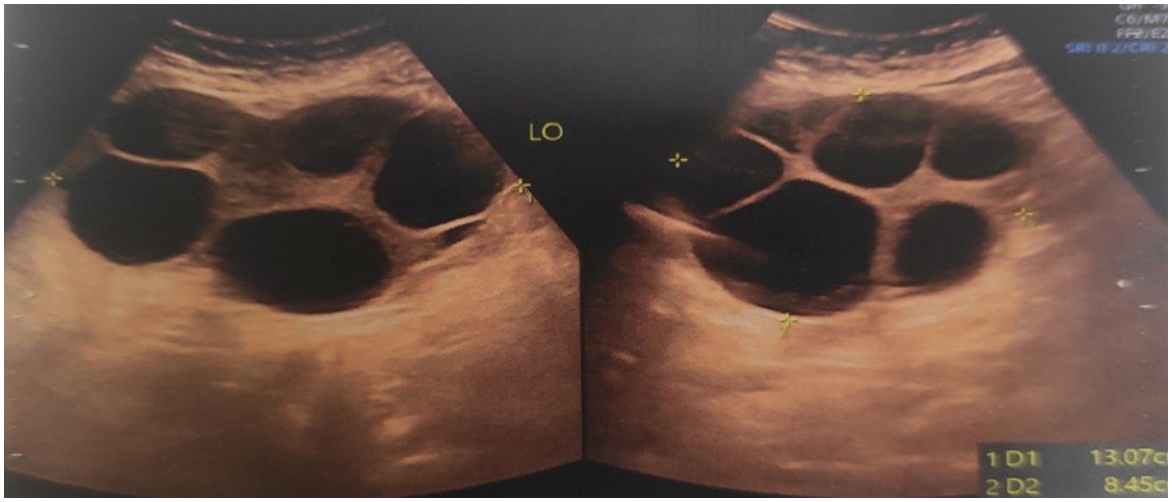
On the 8th postoperative day, the patient came for an outpatient consultation with complaints of bloating, mild abdominal pain, and palpable lump. They had no complaints of nausea, vomiting, or shortness of breath. Ultrasonography revealed bilateral enlarged multicystic ovaries, measuring 13.07×8.45×9.77 cm on the left and 9.02×8.45×5.5 cm on the right, with a small amount of pelvic collection (Figure 1). Serum oestradiol level was 2,582 pg/mL and other laboratory parameters, including haematocrit, serum albumin, liver enzymes, and serum creatinine were normal. The authors diagnosed bilateral multilocular cysts with OHSS. As it was of moderate variety, it was treated on an outpatient basis. The patient was counselled properly regarding treatment and consequences of severe signs/symptoms. Proper intake of fluids and analgesics for pain relief were ensured. The patient came for regular visits with baseline investigations.

With all these measurements, the patient recovered completely. The cysts gradually reduced in size and finally disappeared on Day 42, and hCG reached below cut-off value on Day 72 after D&E.

DISCUSSION

The incidence of OHSS varies for different protocols of infertility treatment. The greater the involvement of stimulation, the higher the incidence becomes. In conventional *in vitro* fertilisation (IVF), approximately one-third of cases may be affected. Moderate to severe OHSS varies from 3.1–8.0%.[4] The 14th European IVF Monitoring Report revealed incidence of hospitalisation due to OHSS is 0.3%.[5] Although OHSS accompanied by natural pregnancy is rare, it can be observed in case of hydatidiform mole

Figure 1: Left-sided multicystic ovary measuring 13.07×8.45×9.77 cm.



where there is cystic proliferation of chorionic villi and release of excess hCG, by which ovaries are hyperstimulated. Hydatidiform mole may be either partial or complete, and most cases worsen after D&E. In the authors' study, OHSS was secondary to complete molar pregnancy with markedly elevated oestradiol level.

In case of OHSS, there is increased production of pro-inflammatory mediators such asVEGF.[2,3] Increased vascular permeability leads to accumulation of fluid in the third space, manifested by dehydration, hypovolaemia, oliguria, haemoconcentration, reduced osmolality, electrolyte imbalance (including hyponatraemia and hyperkalaemia), ascites, and pleural or pericardial effusion. In severe cases, there is a loss of 20% of calculated volume.[6] In the authors' study, they diagnosed the case as moderate OHSS. There was no ascites or pleural effusion due to low BMI (17.71 kg/m²), and intrabdominal pressure was high as larger cysts occupied the abdominal cavity and suppressed leakage of fluids.

Sizes of ovarian cysts are variable. Suzuki et al.[7] reported the largest size of a 30 cm cyst, which normalised within 1 month. In this study, the authors found bilateral enlarged multicystic ovaries measuring 13.07×8.45×9.77 cm on the left and 9.02×8.45×5.5 cm on the right, which returned to normal size on Day 42 after D&E. The duration of reduction of size is not always related to size, as a larger cyst may become normal in a short time, but a smaller cyst may need more time.

Treatment is mostly conservative, as in mild to moderate cases, outpatient management is appropriate. Proper counselling is needed regarding consequences of disease, signs of severity, maintenance of proper hydration, analgesics for pain relief, and regular follow-up with baseline investigations.

Clinicians should be aware of signs and symptoms of severity, which should be explained properly so that the patient can seek medical treatment earlier.

Signs of severity are increasing abdominal distension, shortness of breath, hypotension, tachycardia, reduced urinary output (<1,000 mL/24hrs), weight gain and increased abdominal girth, and increasing haematocrit (>45%). Females with severe OHSS are at risk of thromboembolism, and thromboprophylaxis should be prescribed. Prophylactic anticoagulation by warfarin or low molecular weight of heparin is indicated. Paracentesis may be needed for tense ascites. In the authors' study the patient was treated conservatively with appropriate counselling and care, and recovered completely.

CONCLUSION

Spontaneous onset of OHSS can be a possible complications of hydatidiform mole. The condition worsens after D&E. The clinicians should be aware of ovarian cysts accompanied OHSS. Clinical assessment and timely intervention is helpful to avoid complications. Most of the cases resolve spontaneously and sizes of ovaries become normal even when they are larger.

References

1. Arora R et al. Ovarian hyperstimulation syndrome after a molar pregnancy evacuation. Fertil Steril. 2008;90:1197.e5-e7.
2. Alper MM et al. Ovarian hyperstimulation syndrome: current views on pathophysiology, risk factors, prevention and management. J Exp Clin Assist Reprod. 2009;6:3.
3. Nastri CO et al. Ovarian hyperstimulation syndrome: pathophysiology and prevention. J Assist Reprod Genet. 2010;27 (2-3):121-8.
4. Delvigne A, Rozenberg S. Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. Hum Reprod Update. 2002,8(6):559-77.
5. Kupka MS et al.; European IVF monitoring Consortium, for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. Hum Reprod. 2014:29(10):2099-113.
6. Kumar P et al. Ovarian hyperstimulation syndrome. J Hum Reprod Sci. 2011:4(2):70-5.
7. Suzuki H et al. Ovary hyperstimulation syndrome accompanying molar pregnancy: case report and review of the literature. Arch Gynecol Obstet. 2014:290(4):803-6.

Incidence, Risk Factors, and Aetiologies of Primary Postpartum Haemorrhage After Vaginal Delivery in Kasr Al Ainy University Hospital: A Cross-Sectional Study

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Abstract

Background: Approximately 15% of all pregnancies (about 20 million) suffer from acute severe obstetric complications. The first 24 hours postpartum represent the period with the highest chance of mortality. The most evident complication is haemorrhage, solely accounting for 27% of all maternal deaths worldwide. Haemorrhage occurring postpartum accounts for 73% of all cases. Primary postpartum haemorrhage (PPH) is defined as the loss of at least 500 mL of blood after vaginal delivery or 1,000 mL of blood after Caesarean section within 24 hours of delivery.

Aim: This cross-sectional study aims to evaluate the incidence and determine risk factors of PPH after vaginal delivery in Kasr Al Ainy University Hospital, Cairo, Egypt.

Methodology: The study is a multivariate cross-sectional study. Single population proportion formula was used to determine the sample size in accordance with inclusion and exclusion criteria. Data was collected via assisted questionnaires, and results were statistically interpreted with a confidence interval of 95% to gather the odds ratio (OR) of significant risk factors of PPH. The SPSS® (IBM, Armonk, New York, USA) statistical package was used.

Results: PPH incidence in Kasr Al Ainy in the period of this study was 8.1%. Atonic factors were implicated in 67% of all PPH after vaginal delivery. Most evident risk factors were prolonged labour (OR: 5.1), history of previous PPH (OR: 4.25), hypertension (OR: 2.53), and age ≥35 years (OR: 2.29), respectively.

Conclusion: The authors' research concluded that most cases of PPH were mainly of atonic causes, with prolonged labour being the most evident risk factor.

Key Points

1. Approximately 15% of all pregnancies (about 20 million) suffer from acute severe obstetric complications.

2. The most evident complication is haemorrhage, solely accounting for 27% of all maternal deaths worldwide, with postpartum haemorrhaging accounting for 73% of all cases.

3. This cross-sectional study aims to evaluate the incidence and determine risk factors of primary postpartum haemorrhage after vaginal delivery in Kasr Al Ainy University Hospital, Cairo, Egypt.

BACKGROUND

Every year, over 300,000 females die due to complications in pregnancy and childbirth, and approximately 15% of all females who are pregnant (around 20 million) have acute severe obstetric complications, including haemorrhage.[1,2] The first 24 hours postpartum, and the first week after labour, represent the main periods with the highest chances of mortality. The most evident postpartum complication is haemorrhage, which is the leading cause of maternal mortality in developing countries, accounting for 27% of all maternal deaths that occur worldwide.[3]

As Vlassoff et al.[4] wrote: "Of the three types of haemorrhage—antepartum, intrapartum and postpartum—the postpartum type is by far the most important, accounting for 73% of all hemorrhage cases."

Primary postpartum haemorrhage (PPH) is formally defined as the loss of at least 500 mL of blood after a vaginal birth, or at least 1,000 mL of blood after a Caesarean section within 24 hours of delivery, or any blood loss that may lead to hemodynamic instability.[5]

Postpartum complications are substantially influenced by multiple factors and variables, which range from socioeconomic status to the level of patient education, and access to proper healthcare and pregnancy follow-up, reaching to as late as the mode of delivery. Each risk factor can be linked directly to one of the established aetiologies of PPH, the four Ts: tone: uterine atony; tissue: retained products; trauma: uterine or vaginal injuries; and thrombin: bleeding disorders. A low socioeconomic status, illiteracy, and poor access to healthcare can lead to lack of antenatal care visits, which in turn leads to unnoticed antenatal problems like hypertension, which can predispose to uterine atony. In developing countries, an estimated 70% of females do not receive postpartum care, and some 60–80% of maternal deaths occur during this time period.[2]

To be able to identify these risk factors is to be able to prevent the morbidities and mortalities related to them. In Egypt specifically, according to demographic and health surveys, maternal mortality ratios were reduced from 84 to 52 deaths per 100,000 live births between 2000–2013.[4] On a more specific note, data from this source conclude that the percentage of maternal deaths attributable to PPH declined from 34% in 2000 to 20% in 2013.[4]

AIM AND OBJECTIVES

The aim was to study the incidence and aetiology of PPH and their indices through measuring the incidence of postpartum haemorrhage after vaginal delivery in Kasr Al Ainy University Hospital, Cairo, Egypt, and determining the most common risk factors and aetiologies of postpartum haemorrhage in Kasr Al Ainy University Hospital.

Study Design

The authors conducted a cross-sectional study of postpartum haemorrhage after vaginal delivery in Kasr Al Ainy University Hospital, from the 1st November 2019. Postpartum haemorrhage is defined as blood loss exceeding 500 mL following vaginal delivery.

Study Setting
The study was carried out in the Obstetrics and Gynecology Emergency Department and the Postpartum Ward in Kasr Al Ainy University Hospital between 1st November 2019–15th February 2020.

The purposive selection of this hospital was based on its characteristics of being a large referral teaching hospital in the public sector with qualified health professionals, and a high caseload to mirror the population as best as possible. It is the main referral and teaching tertiary hospital in Greater Cairo, and is affiliated with the Faculty of Medicine at Cairo University.

Study Sample
In Kasr Al Ainy, the total number of vaginal deliveries in 2018 was 7,605, with an average of 20.8 deliveries per day. Therefore, the number of deliveries throughout the 106 days of data collection in the period from 1st November 2019–15th February 2020 was estimated to reach approximately 2,204.

Single population proportion formula was used to determine the sample size with the following assumptions: a confidence level of 95%, a margin of error of 5%, and a calculated global incidence of PPH after vaginal delivery of 10.8%.[6] The sample size was n=139, which was then sampled using non-probability purposive sampling.

Inclusion Criteria
Inclusion criteria of this research included vaginal delivery with any presentation and any gestational age (preterm, term, post-term), multi-foetal pregnancy, vaginal birth after Caesarean section, live or still birth, and coagulopathies.

Exclusion Criteria
Exclusion criteria included performed Caesarean sections, abortion, and patients presenting to the emergency department with postpartum haemorrhage who gave birth at another hospital.

METHODOLOGY

Ethical Approval and Consent
The study protocol was approved by Cairo University's ethical committee and review board. All participating patients provided informed consent for the collection of data.

Data Collection
The research team was responsible for carrying out the data collection, with the help of the attending residents, in a retrospective manner. Data collectors undertook a daily review of all admissions to labour, delivery, postpartum, and the emergency department to identify all eligible patients.

Patients' charts were reviewed to retrieve the diagnosis of primary PPH. The amount of bleeding, if present, was estimated visually, as per World Health Organization (WHO) recommendations,[2] and clinically[2] (change in vital signs by >15% or heart rate ≥110 beats/minute, blood pressure ≤85/45 mmHg, O_2 saturation <95%; California Maternal Quality Care Collaborative [CMQCC] staging system) by the resident to determine whether it was within normal limits, or classified as postpartum haemorrhage. Table 1 used data collected from the patients, using an assisted questionnaire translated to Arabic.[7]

DATA ANALYSIS

This study is a multivariate, cross-sectional study. Relative and absolute risks could not be calculated because of the multiple variables at place that can co-occur simultaneously or separately.

Confidence interval (CI) of data analysis was specified at 95%, making the margin of error (p=0.05) and its critical value determined to be 1.96, which is traditionally chosen for medical studies with p=0.05.[8] The authors used a sample size for a single proportion formula calculator to calculate the necessary sample size.[9] "This calculator uses the following formula for the sample size n: n=N*X / (X+N–1) where X=Zα/22 *p*(1-p)/MOE2, and Zα/2 is the critical value of the normal distribution at α/2 (e.g., for CI of 95%,

Table 1: Incidence of primary postpartum haemorrhage in this study.

Incidence of PPH in this study	$\frac{\textit{(Patients who had PPH)}}{\textit{(Total number of patients)}}$	x100
% contribution of specific (e.g., atonic) causes in the total number of cases	$\frac{\textit{(Patients who had PPH from a specific cause)}}{\textit{(Total number of patients who had PPH)}}$	x100
Mean	$\frac{\text{(Sum of cases)}}{\text{(Number of cases)}}$	
%	$\frac{\text{(Number of specific event)}}{\text{(Total number)}}$	x100
Odds of each risk factor (exposed group)	$\frac{\text{(Patients with risk factor with PPH)}}{\text{(Patients with risk factor without PPH)}}$	
Odds of non-exposed group	$\frac{\text{(Patients without risk factor with PPH)}}{\text{(Patients without risk factor without PPH)}}$	
Odds ratio of each risk factor	$\frac{\text{(Odds of the event in the exposed group)}}{\text{(Odds of the event in the non–exposed group)}}$	

PPH: primary postpartum haemorrhage.

α is 0.05 and the critical value is 1.96), MOE is the margin of error, p is the sample proportion (the global incidence of vaginal PPH is calculated to be 10.8%), and N is the population size."[9]

A finite population adjustment has been applied to be the estimated number of deliveries throughout the period of the study (106 days [1st November 2019–15th February 2020], with an average of 20.8 deliveries/day; a finite population of 2,204 deliveries throughout the period of the study was determined).

RESULTS

The sample size required for the multivariate study with a CI of 95% was calculated to be at least 139 patients. This study included 554 patients in total, with the following population characteristics: mean maternal age: 26.6 years; 82.6% of all the participants presented with gestational age between 38–42 weeks; and 87.8% of the neonatal birth weight was between 2.5–4.0 kg (Table 1).

Population Characteristics of this Study

Incidence of PPH in this study: patients with PPH (45)/total number of patients (554) x100. The Incidence of PPH in this study was 8.12%.

Of all the risk factors, the most statistically significant of PPH ($p<0.05$) were all atonic causes, primarily prolonged labour (odds ratio [OR]: 5.10), history of previous PPH (OR: 4.25), hypertension (OR: 2.53), and lastly maternal age ≥35 years (OR: 2.29). Atony alone contributed to 67% of PPH, and atony with trauma contributed to 13% of PPH. Other aetiologies attributing to PPH were retained tissues (8%), bleeding disorders (8%), and traumatic causes (4%; Figure 1 and Figure 2).

DISCUSSION

PPH indices and risk factors differ from one country to another, and from one institution to another, depending on the facilities, skills of the doctors, and education of the public. The authors made a deliberate decision to conduct their study in a tertiary center in Egypt providing public healthcare, to be able to have a wholesome vision of risk factors and causes of PPH implicated in the mass public. To be able to identify these

Figure 1: Contribution of major primary postpartum haemorrhage aetiologies in this study.

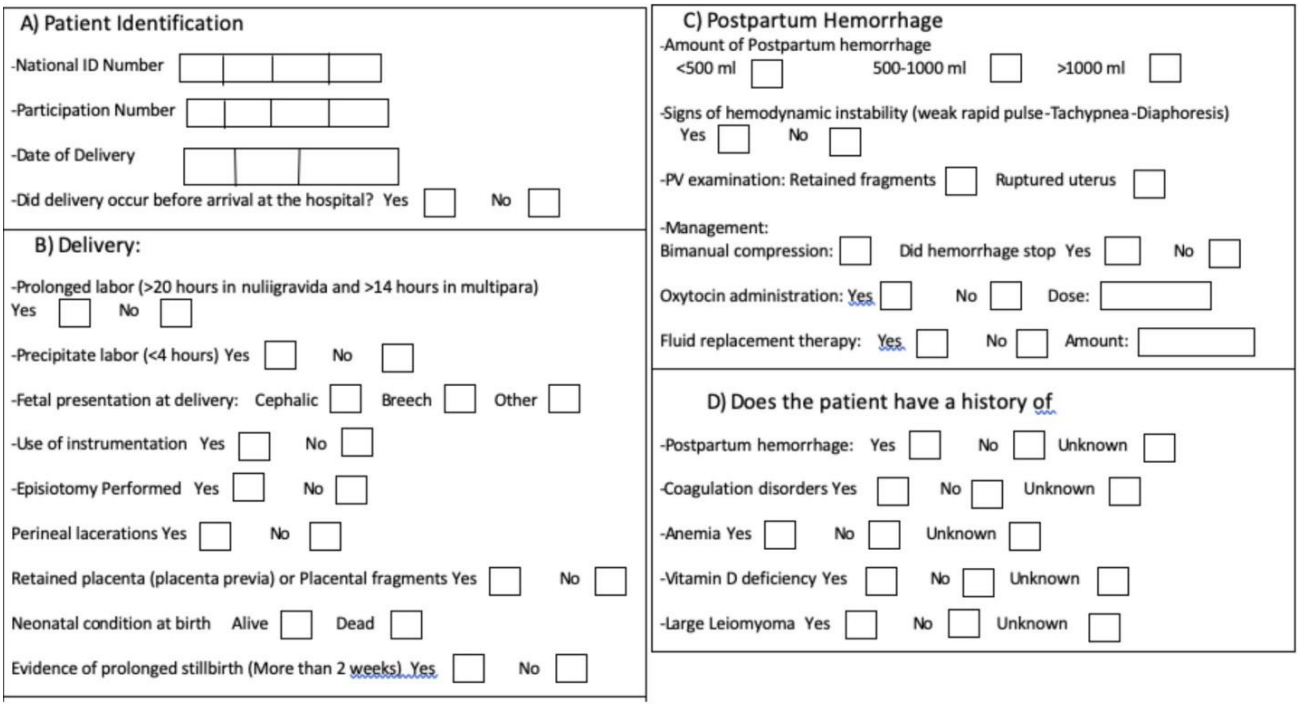
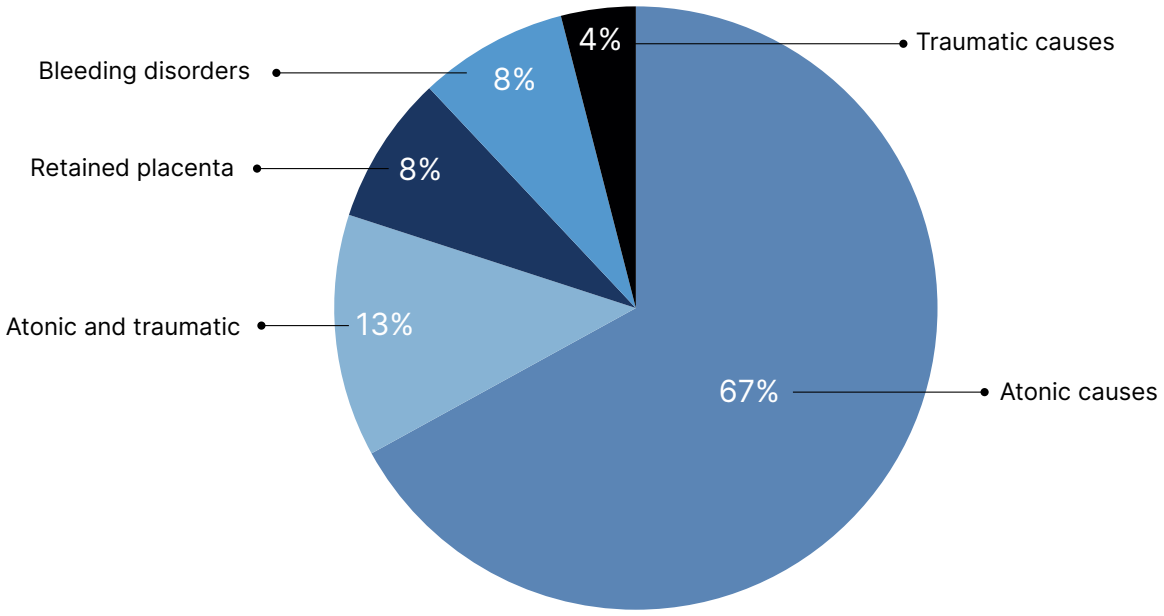


Figure 2: Major risk factors.



Major PPH causes identified in this study. Atonic causes were implicated in 67% of the cases studied; atonic and traumatic (13%); retained placenta and bleeding disorders (8%); and solely traumatic causes (4%).

PPH: primary postpartum haemorrhage.

risk factors and aetiologies and tackle them will ultimately decrease the incidence of PPH.

PPH incidence in Kasr Al Ainy in the period of this study was 8.1%, which is lower than the calculated worldwide incidence of PPH after vaginal delivery (10.8%).[6] Adding to that, it was still less than the incidence of PPH in Egypt calculated in 2013 (20%), which in turn was already less than the incidence calculated in Egypt in 2000 (34%).[4] This may reflect the continuous strive to tackle risk factors of PPH, and decrease PPH incidence.

The leading risk factors identified in this study: prolonged labour, history of previous PPH, hypertension, and maternal age ≥35 years, all contribute to uterine atony as the most common aetiology of PPH. Out of all the cases studied, atony alone contributed to 67% of PPH, and atony with trauma contributed to 13% of PPH. Prolonged labour in this study was diagnosed as patients whose labour lasted over 20 hours for primigravida, and more than 14 hours in multipara, who still gave birth vaginally. Patients with prolonged labour who eventually underwent Caesarean section were excluded from the study. This highlights the urgency in tackling atony as the commonest aetiology of PPH in the authors' region.

One noticeable aspect in this study is that bleeding disorders were attributed to 8% of the cases of PPH. Compared with the worldwide percentage of bleeding disorders causing 3% of PPH cases,[10] this seems to be quite high. This can be linked to the fact that with few antenatal care visits, females with bleeding disorders are neither diagnosed, nor adequately treated before giving birth. This highlights the weight of lack of antenatal care visits as a risk factor to PPH, and provides insight to how risk factors proposed attribute to aetiologies of PPH.

The authors chose Kasr Al Ainy specifically because it is one of the largest tertiary centres in Egypt, and offers public healthcare. To avoid the bias of the tertiary center receiving referred cases, the authors excluded the patients who gave birth somewhere else and presented with established PPH. They were also able to surpass the sample size calculated (n=139), and reached 554 participants, further minimising the margin of error. On the other hand, it being the largest tertiary center might be at the advantage of having the best trained residents to deal with obstetric complications, and may not, therefore, reflect the true incidence of PHH in Egypt. However, by pointing out the most statistically significant risk factors ($p<0.05$) with the highest OR, those risk factors are likely be the most significant ones in other areas in Egypt and many more developing countries. With their identification, appraisal of ways to overcome these risk factors will decrease the incidence of PPH. The authors also compared their results and OR with other studies done elsewhere to test the relevance of their data.

Looking at similar studies conducted in different parts of the world, Africa had the lion's share of PPH compared with other continents, with an incidence of 25.7%. Second was North America with PPH incidence of 13.1%, then Europe with 12.7%, Asia 8.5%, South America 8.2%, and Australia 7.2%.[6]

Even though, at first glance, those values can be shocking, one explanation of these variabilities can be attributed to the difference in the methodology of the studies conducted. Studies that were conducted at tertiary hospitals gave a higher incidence than elsewhere due to the referrals received.

With numerous studies conducted in different demographic areas, one may expect that risk factors would differ from one place to another. However, many studies identified the same risk factors obtained in this study as being the most significant in causing PPH.[11-16] Remarkably, the OR obtained from said studies showed comparable results to the OR derived from the authors' study. This finding serves as a valuable counterbalance to the potential bias associated with this study being conducted in a tertiary centre, with well-trained residents and greater access to interventions.

On the other hand, some studies proposed other risk factors contributing more evidently to PPH, which the authors did not observe in their study: macrosomia,[17,18] use of labour induction or augmentation,[16,17] and multi-foetal gestation.[18,19]

Thus, even though learning how to tackle each risk factor and adopting the recommendations would decrease PPH worldwide, it is still important to identify which risk factors play the biggest role in each demographic area.

STRENGTHS AND LIMITATIONS

Conducting the study in Kasr Al Ainy University Hospital, as one of the biggest tertiary centres in Egypt, meant that the authors had an extensive pool of patients. This helped a lot in exceeding the statistically suggested sample, and obtaining a more accurate presentation of the incidences and ORs of the risk factor. This strengthens the study's statistical significance, and enriches the authors' knowledge of the risk factors proposed, hence facilitating the confidence in adhering to the proposed recommendations.

Records were kept, albeit on paper, and the authors were able to identify the study sample in accordance with the inclusion and exclusion criteria; this helped in the follow-up of blood loss and management.

On the other hand, the authors were aiming to continue data collection for a longer period to obtain even more inclusive data, but this came to a halt due to the emergence of the COVID-19 pandemic. Adding to that, with the new emerging studies on the effect of previously contracting COVID-19 with haematological diseases, including coagulopathies, the authors deemed it best to avoid resumption of data collection with the introduction of a new variant in the risk factors.

Another limitation faced was the unavailability of digital records of the patients. To manually go through patient's files and documents with the high caseload put a strain on the authors' timeframe, and decreased the efficiency of the study.

CONCLUSION

The incidence of PPH in Kasr Al Ainy is 8.1%, which, even though is less than the incidence studied in 2013 in Egypt,[4] and less than the worldwide incidence of PPH,[6] is still high. To decrease this incidence, a review of the most statistically significant risk factors was vital. Atonic causes of postpartum dominated the risk factors, most significant of which are prolonged labour, history of previous PPH, hypertension, and advanced maternal age ≥35 years.

RECOMMENDATIONS

To tackle the risk factors as early as possible, antenatal care and antenatal care visits with health education to mothers are of critical importance. This can early on identify high risk patients; stabilise any current comorbidities, including hypertension or coagulopathies; and track the progression of the pregnancy.

Another way is to prevent prolonged labour, which can be done through timely follow-up of the stages of labour of the patient, defining high risk patients, and initiating early intervention when required.

Adherence to active management of the third stage of labour, including routine administration of oxytocin, which can prevent atony; clamping and cutting the umbilical cord immediately after delivery; and controlled cord traction can prevent bleeding.[20]

Other measures that can be adopted are avoiding injurious instrumental deliveries, immediate repair of any vaginal or perineal tears, and making sure there are no products of conception left.

References

1. Glasier A et al. Sexual and reproductive health: a matter of life and death. Lancet. 2006;368(9547):1595-607.
2. World Health Organization (WHO). Maternal mortality in 2000: estimates developed by WHO, UNICEF and UNFPA. Available at: https://apps.who.int/iris/handle/10665/42930. Last accessed: 10 November 2019.
3. Say L et al. Global causes of maternal death: a WHO systematic analysis. Lancet Glob Health. 2014;2(6):e323-33. Available at: https://www.thelancet.com/action/ showPdf?pii=S2214-109X%2814%2970227-X. Last accessed: 10 November 2019.
4. Vlassoff M et al. The cost to the health system of postpartum hemorrhage in Egypt. Guttmacher Institute. 2016. Available at: https://www.guttmacher.org/report/cost-of-postpartum-hemorrhage-in-egypt. Last accessed: 10 November 2019.
5. World Health Organization (WHO). WHO recommendations for the prevention and treatment of postpartum haemorrhage. 2012. Available at: https://apps.who.int/iris/bitstream/handle/10665/75411/9789241548502_eng.pdf. Last accessed: 31 May 2022.
6. Calvert C et al. Identifying regional variation in the prevalence of postpartum haemorrhage:

a systematic review and meta-analysis. PLoS One. 2012;7(7):e41114.

7. Daif AH et al. Postpartum hemorrhage (responses). Available at: https://docs.google.com/spreadsheets/d/10JnP0u5gTB2d1BnvxH70qSHukybWj0yeXqaX1i40LXM/edit#gid=943222552. Last accessed: 10 November 2019.
8. Tenny S, Hoffman MR. Odds ratio (2023) Treasure Island: StatPearls. Available at: https://www.ncbi.nlm.nih.gov/books/NBK431098/. Last accessed: 10 Nov 2019.
9. Select Statistical Consultants; Brooks S. Population proportion - sample size. 2018. Available at: https://select-statistics.co.uk/calculators/sample-size-calculator-population-proportion/. Last accessed: 20 February 2023.
10. Collins P et al. Management of coagulopathy associated with postpartum hemorrhage: guidance from the SSC of the ISTH. J Thromb Haemost. 2016;14(1):205-10.
11. Firmin M et al. Postpartum hemorrhage: incidence, risk factors, and causes in western French Guiana. J Gynecol Obstet Hum Reprod. 2019;48(1):55-60.
12. Ngwenya S. Postpartum hemorrhage: incidence, risk factors, and outcomes in a low-resource setting. Int J Womens Health. 2016;8:647-50.
13. Oyelese Y, Ananth CV. Postpartum hemorrhage: epidemiology, risk factors, and causes. Clin Obstet Gynecol. 2010;53(1):147-56.
14. Kebede BA et al. Prevalence and predictors of primary postpartum hemorrhage: an implication for designing effective intervention at selected hospitals, southern Ethiopia. PLoS One. 2019;14(10):e0024579.
15. Kramer MS et al. Incidence, risk factors, and temporal trends in severe postpartum hemorrhage. Am J Obstet Gynecol. 2013;209(5):448.e1-7.
16. Prata N et al. Inability to predict postpartum hemorrhage: insights from Egyptian intervention data. BMC Pregnancy Childbirth. 2011;11:97.
17. El Badawy A et al. Assessment of risk factors for primary postpartum hemorrhage at Zagazig University Hospitals. Zagazig Univ Med J. 2017;23(2):1-9.
18. Sosa CG et al. Risk factors for postpartum hemorrhage in vaginal deliveries in a Latin-American population. Obstet Gynecol. 2009;113(6):1313-19.
19. Ononge S et al. Incidence and risk factors for postpartum hemorrhage in Uganda. Reprod Health. 2016;13:38.
20. Rath W. [Active management of the third stage of labour (AMTSL) - the end of a 50 years-dogma?]. Z Geburtshilfe Neonatol. 2013; 217(5):173-6. (In German).

An Unusual Clinical and Radiological Presentation of Ovotesticular Disorders of Sex Development with Male and Female External Genitalia: A Case Report

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Disclosure: Ethical approval was taken from Haroon Khan, Head of Department of Radiology at the Punjab Institute of Neurosciences (PINS), Lahore, Pakistan. The patient and their parents signed a written informed consent form. Written informed consent was taken from the patient and their parents for the publication of this case and the relevant radiological images. All relevant images have been uploaded along with the manuscript. This study complied with the latest version of the Helsinki Declaration. The authors have declared no conflicts of interest. No funding was received for this study. Tahir Khan conducted the literature review, drafted initial document, created images, and amended the final draft. Yasin and Haroon Khan oversaw the research and helped with revision. Raman revised the manuscript and edited images. The final version of the manuscript was approved by all authors.

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Abstract

Background: Ovotesticular disorders of sex development (ODSD), previously known as true hermaphroditism, is a rare disorder of sexual differentiation that causes ambiguity in external genitalia and the presence of both ovarian and testicular elements in the gonads. The condition is characterised by the existence of testicular tissue with distinctive tubules and ovarian tissue in the same gonad (ovotestis), or separately in an individual. The most common cytogenetic karyotype is 46,XX. The age and presentation of symptoms vary, but typically include hypospadias, urogenital sinus, inguinal hernia, cryptorchidism, and gynaecomastia during the peripubertal age, as well as lower abdominal mass.

Case Presentation: A 15-year-old female exhibited typical male genitalia, including a normal penile shaft, scrotal sac, palpable testes, and a urethral opening, with a

separate vaginal opening located between the penile shaft and scrotum. Pelvic MRI confirmed the presence of both testes within the scrotal sac and an infantile-sized uterus with a central endometrium, as well as a left-sided ovary in the pelvic region. The right ovary was not seen. Correlative ultrasound confirmed these findings and indicated that the testes had a normal echo texture and vascularity. The patient was diagnosed with ODSD, and further hormonal testing was recommended.

Conclusions: ODSD is a rare cause of genital ambiguity. Typically, both paramesonephric and mesonephric duct derivatives are seen, and most of the affected individuals present as neonates or infants with ambiguous external genitalia. At peripubertal age, the symptoms typically include hypospadias, urogenital sinus, inguinal hernia, unilateral or bilateral cryptorchidism, and gynaecomastia. Fertility is not uncommon and has been reported in female-reared patients. The ovotestis is the commonest gonad in people with ODSD and are known to be predisposed to germ cell tumours. Over 500 cases of ODSD have been reported in the literature. Ultrasound is the first line of investigation used to see the pelvic organs. The non-invasive test of choice for detecting paramesonephric and mesonephric duct derivatives is an MRI pelvis. The purpose of this paper is to describe various clinical and radiological features associated with ODSD.

Key Points

1. A rare congenital disorder called ovatesticular disorders of sex development (ODSD) is characterised by the co-existence of ovarian and testicular tissue in the same person. It is frequently accompanied with ambiguous external genitalia, and can manifest in a variety of ways with a range of symptoms, including gynecomastia, hypospadias, urogenital sinus, inguinal hernia, and cryptorchidism.

2. A combination of clinical, radiographic, and hormonal tests is frequently used to diagnose ODSD. To see the reproductive organs within and find paramesonephric and mesonephric duct derivatives, ultrasound and MRI are useful imaging tools. Hormone testing aids in evaluating hormone levels and choosing the best course of action.

3. Endocrinologists, geneticists, paediatric surgeons, and psychologists are required to manage ODSD, and treatment options include hormonal therapy to induce secondary sexual characteristics consistent with the patient's gender identity, and surgical interventions to address ambiguous external genitalia. Long-term follow-up care, including hormone levels, bone health, and psychosocial support, is essential for people with ODSD.

INTRODUCTION

Ovotesticular disorders of sex development (ODSD) is an extremely rare congenital disease characterised by the presence of both testicular and ovarian tissue in the same gonad or person.[1] In the literature on ODSD, approximately 500 cases have been reported.[2] Hungerford et al.[3] reported the first chromosomal findings in ODSD in 1959, when they discovered a 46,XX chromosomal complement in an individual's peripheral blood lymphocytes with the disorder. ODSD is a genetically heterogeneous condition, with 46,XX being the most common karyotype. Depending on the amount of testicular tissue present, the phenotype of the external genitalia can range from normal male to normal female.[1] The majority of ODSD cases have a normal female karyotype.[4]

CASE PRESENTATION

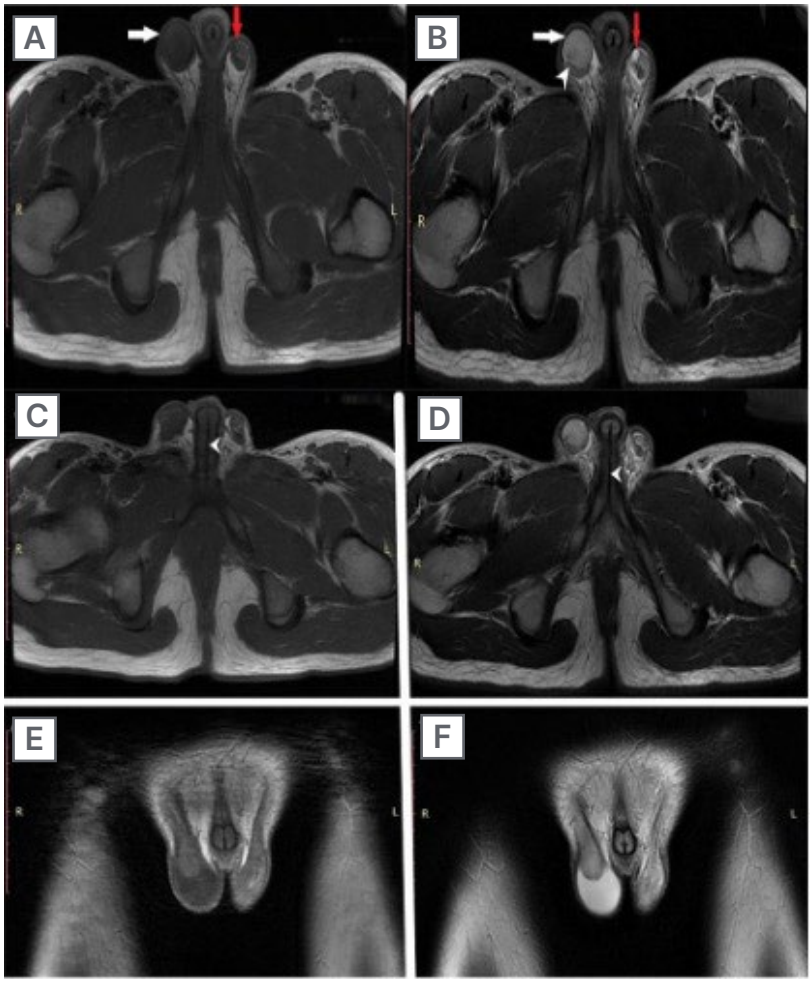
A child born from a consanguineous marriage who was reared as a female and is now 15 years old demonstrated a slow emergence of symptoms, such as a deepening of voice, increasing facial hair, and primary amenorrhoea.

Tanner Stage III pubic hair development and Tanner Stage II breast development were seen in the patient. A penile shaft with a regular urethral entrance, a scrotal sac with normally positioned palpable testes, and a separate vaginal opening between the penile shaft and scrotum were all found on physical examination. Bilateral testes were totally descended and non-retractile. Following an investigation, it was discovered that the patient had a non-institutional unsupervised delivery at home, and so no relevant paperwork detailing newborn assessment was accessible. No other family member had a comparable condition. Prader staging was not included in the referring physician's clinical evaluation.

Pelvis MRI

MRI pelvis scans showed both testes within the scrotal sac with the right testis measuring 28×15×17 mm along with mild hydrocele. The left testis measured 28×15×19 mm. There was a small left-sided epididymal cyst measuring 6.7 mm in diameter (Figure 1A and B). The penile shaft and glans were visualised with normal-appearing corpora cavernosa and corpora spongiosum (Figure 2). Prostate or seminal vesicles are not appreciated at their anatomical location.

Figure 1: MRI showing the testicles of a 15-year-old individual with ovotesticular disorders of sex development, who presented with deepening of voice and primary amenorrhoea.



A and **B**) T2 and T1-weighted images showing testicles (white arrow), right-sided mild hydrocele (white arrowhead), and small epididymal cyst on left side (red arrow). **C** and **D**) Axial T1 and T2-weighted images showing the urethral opening (white arrow heads). **E** and **F**) Coronal T1 and T2-weighted images depicting scrotal sac with testicles.

Within the pelvis, there is an infantile-sized uterus measuring 24×12 mm in axial dimensions (Figure 2A and B). Central endometrium is appreciated measuring about 3.8 mm in thickness. The multi-cystic structure is seen in the left adnexal region conforming to the features of the ovary, measuring 25×19×14 mm (Figure 2). The right ovary was not seen.

The uterine cavity continues inferiorly with the vagina, which has an external perineal opening. Within the perineum, the urethra opening into penile shaft is seen anteriorly with a separate vaginal opening posterior to it. Anal canal and anal verge were visualised separately (Figure 2D).

Greyscale and Doppler Ultrasound

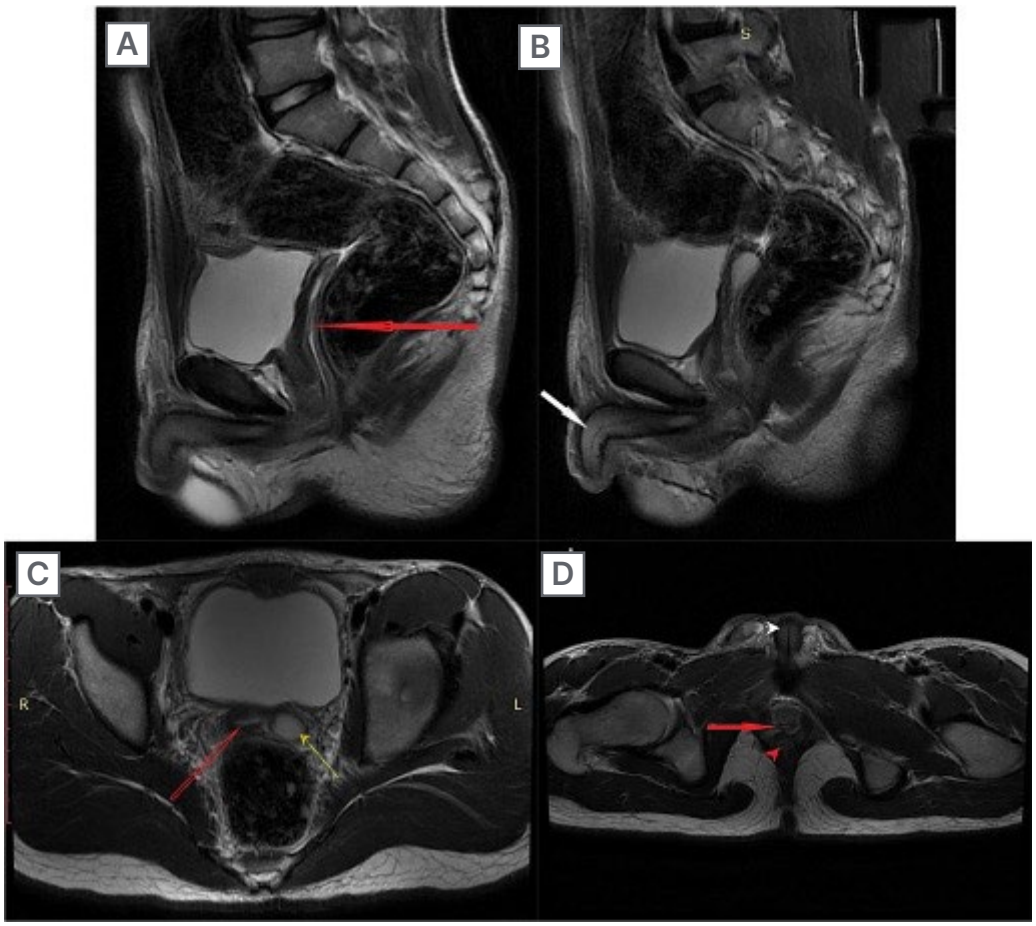
A high frequency linear probe was used to perform an ultrasound of inguinoscrotal region. It revealed both testes with normal echotexture and normal blood flow on colour doppler (Figure 3).

Biochemistry

Hormonal assessment was done resulting in the following parameters (Table 1).

No tissue was sampled for histopathological confirmation. Karyotyping was advised but could not be performed due to the lack of availability

Figure 2: MRI showing the uterus with endometrium and penile shaft of a 15-year-old individual with ovotesticular disorders of sex development, who presented with deepening of voice and primary amenorrhoea.



A and **B**) Sagittal T2-weighted images showing the uterus with endometrium (red arrow in **A**) and penile shaft (white arrow in **B**). **C**) Axial T2-weighted image showing the uterus with endometrium (red arrow) and left sided ovary (yellow arrow). **D**) Axial T2-weighted image showing vagina (red arrow), anal canal (red arrowhead), and penile shaft with urethra (white arrowhead).

Figure 3: A greyscale doppler ultrasound of scrotum.

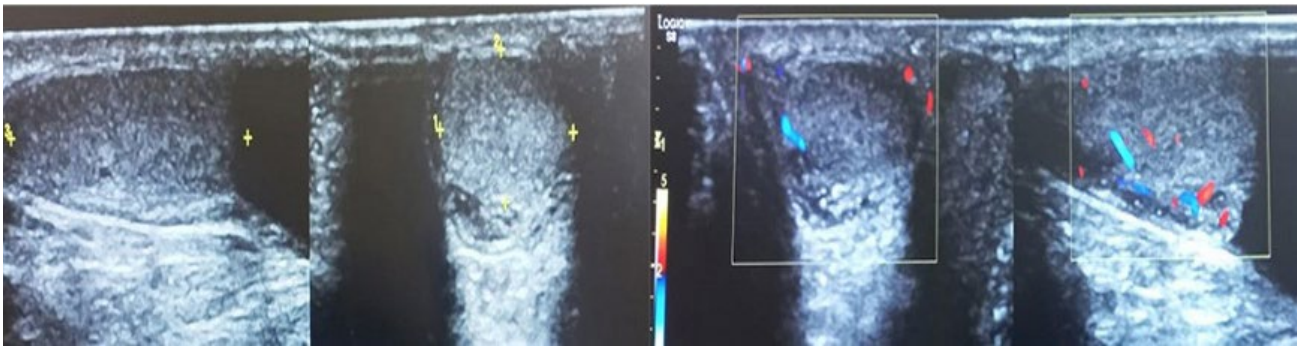


Table 1: Hormonal assessments.

Tests	Results	Reference range
Prolactin	16.74 ng/dL	Male: 3.0–14.7 ng/mL Female: 3.8–23.0 ng/mL
FSH	50.70 mIU/ mL	For both genders: 15–18 years; 0.7–9.6 mIU/mL
LH	5.34 mIU/ mL	Male: 14–18 years; 1.3–9.8 mIU/mL Female: 14–18 years; 0.5–41.7 mIU/mL
Testosterone	5.85 ng/dL	Male: 14–18 years; 0.48–21.8 ng/dL Female: 14–18 years; 0.13–1.08 ng/dL

FSH: follicle-stimulating hormone; LH: luteinising hormone.

at the authors' institution. Thus, on basis of the clinical, radiological, and hormonal investigations, a diagnosis of ODSD was made.

DISCUSSION

Disorders of sexual development (DSD) is an umbrella term that refers to any problem in which the genital organ is atypical in relation to chromosomes or gonads. The most common causes of DSD are congenital adrenal hyperplasia and mixed gonadal dysgenesis, which account for more than half of all cases.[5] The most uncommon form of DSD is ODSD, in which the external genitalia are ambiguous and the gonads contain both testicular and ovarian elements.[6] The gonads can contain any combination of ovary, testes, or both (ovotestis).[5] This anomaly can be caused by a variety of mechanisms, the most common of which is mosaicism or chimerism. Increasing maternal age is known to increase dizygotic twinning and, as a result, the risk of chimerism.[6]

The underlying karyotype frequently influences clinical presentation. In ODSD, the most common karyotype is 46,XX.[7] The presentation features are variable. It also includes inguinal hernia or gynaecomastia, in addition to ambiguous genitalia. An ovary performs better than a testis in terms of functionality. The leydig cell function of the dysgenetic testis is generally insufficient, but ODSD may be well virilised, as in the authors' case. Fertility is not uncommon and has been reported in female patients.[2]

In ODSD, the risk of gonadal neoplasm ranges from 2.6–4.6%, which is lower than in other DSD disorders.[2] Ovotestis is the most common gonad in patients with ODSD, and these gonads are known to be predisposed to germ cell tumours.[1] The most common histologic subtype of these tumours is dysgerminoma.[2]

In the literature, over 500 cases of ODSD have been reported. An MRI pelvis is the non-invasive test of choice for detecting paramesonephric and mesonephric duct derivatives. The pelvic organs can also be visualised using ultrasound.[2] Additionally, radiology is important in the management of ODSD because it provides information on the presence and abnormalities of internal reproductive organs. Radiological studies, particularly ultrasound and MRI, assist clinicians in making an accurate diagnosis, determining the extent of the condition, and planning treatment options. As a result, radiology is an essential part of the diagnostic workup and treatment plan in patients with ODSD.

Differentiating ODSD from other illnesses with comparable clinical characteristics is critical for effective treatment. Androgen insensitivity syndrome, congenital adrenal hyperplasia, and mixed gonadal dysgenesis are the key differential diagnosis to examine. Androgen insensitivity syndrome is defined by individuals with a female phenotypic but a male karyotype (46,XY). These people are resistive to androgen activity, resulting in inadequate virilisation of the external genitalia.[8] Congenital adrenal hyperplasia, on the other hand, is caused by enzyme defects in the adrenal steroid production. It can cause virilisation of the external genitalia in people who are born female. To distinguish congenital adrenal hyperplasia from ODSD, a complete clinical examination, hormone study, genetic testing, and radiological imaging are required.[9] Mixed gonadal dysgenesis can manifest with a wide range of phenotypic characteristics, including ambiguous genitalia, and is distinguished by the presence of a streak gonad, a nonfunctional gonad, as well as a dysgenetic testis or ovary.[9]

To achieve the best results, ODSD requires a multidisciplinary approach, comprising of endocrinologists, geneticists, paediatric surgeons, and psychologists. Hormonal therapy and surgical treatments are two treatment possibilities. Hormonal therapy attempts to instil secondary sexual characteristics that are consistent with the patient's gender identity. When a female patient is assigned, oestrogen replacement medication can be started. Testosterone replacement therapy may be considered for male patients. Gonadotropin-releasing hormone analogues may also be used to postpone puberty, and to give the patient time to make informed decisions about their gender identity.[10] Surgical operations are adapted to the demands and preferences of the individual. Gonadectomy (removal of gonadal tissue) is commonly advised to lower the risk of cancer and to prevent hormonal abnormalities. After thorough examination of aspects such as fertility potential, anatomical traits, and patient preferences, the decision on which gonad to keep (ovary or testis) is taken. To correct the ambiguous external genitalia and provide a more coherent appearance, reconstructive procedures may be undertaken.[11] After adequate counselling, the authors' patient was referred to their physician for further management.

Individuals with ODSD require long-term follow-up care. To guarantee optimal health outcomes, hormone levels, bone health, and overall physical well-being, patients with ODSD must be monitored. Furthermore, psychosocial support is important in assisting patients and their families in coping with the difficulties associated with DSD. Individuals can benefit from support groups, counselling, and psychological interventions throughout their journey.[12]

CONCLUSION

Finally, the case report presented here demonstrates an unusual radiological presentation of ODSD with both external genitalia. The presence of testicular tissue with recognizable tubules as well as ovarian tissue in the same individual led to the diagnosis of ODSD. This case emphasises the significance of identifying paramesonephric and mesonephric duct derivatives using non-invasive diagnostic tools, such as pelvic MRI and ultrasound. Even though ODSD is a rare condition, it should be considered as a possible cause in neonates, infants, and peripubertal individuals of genital ambiguity. Understanding the clinical and radiological review of ODSD can help to ensure

that affected individuals are managed in a timely and appropriate manner.

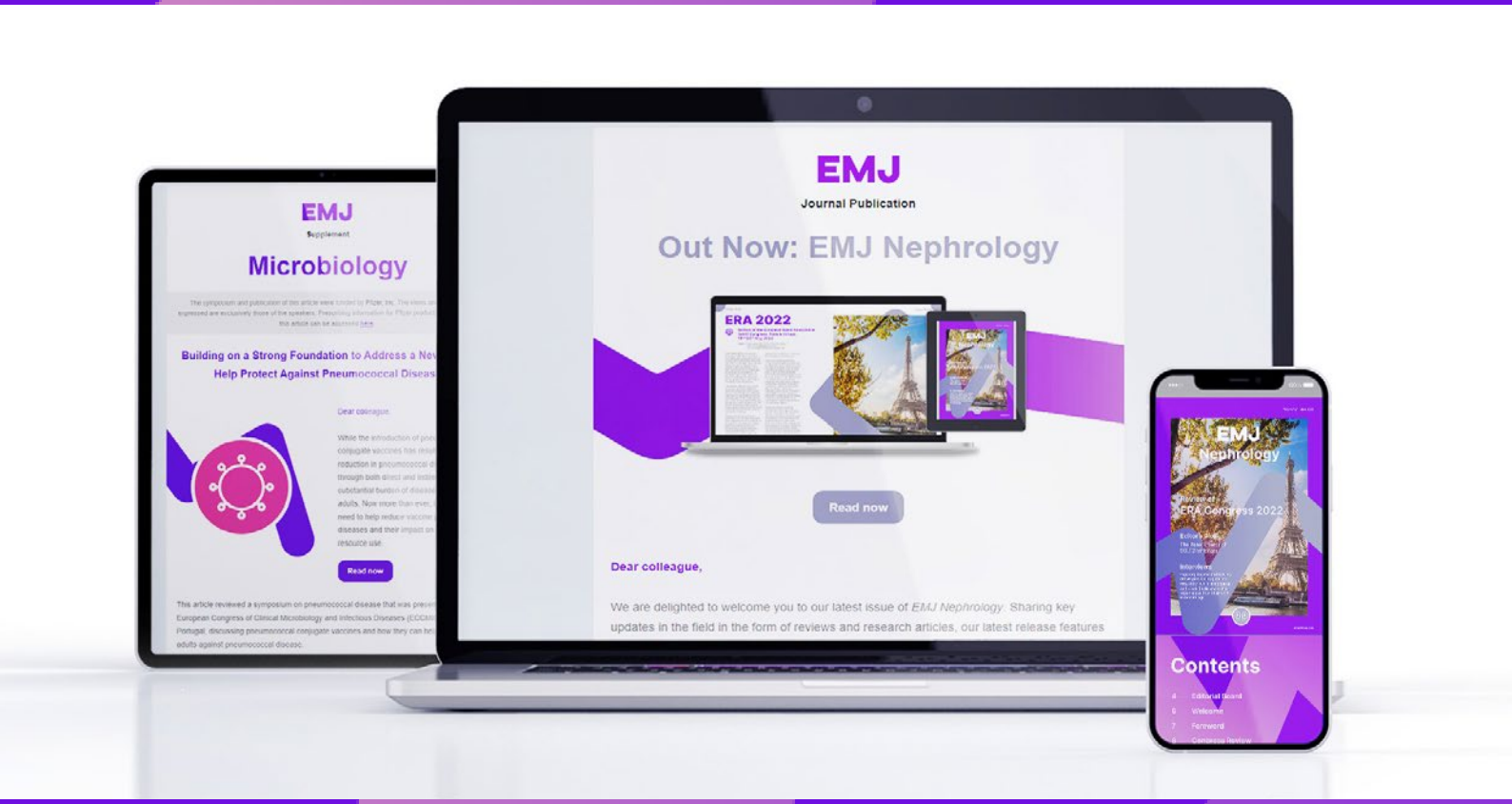
LIMITATIONS OF THE CASE REPORT

Genetic testing, such as karyotyping and molecular testing, is critical for detecting and distinguishing various DSD problems. The case report's lack of specific genetic study data impedes a comprehensive understanding of the underlying genetic disorders that contribute to ODSD. Histopathological examination of gonadal tissue is also required to demonstrate the existence of ovarian and testicular elements. There may be a lack of precise histopathological descriptions or the inclusion of histological picture in the case report, which could provide additional insights into the specific histological abnormalities identified.

When reading the case report's findings and conclusions, it is critical to keep these limitations in mind. More research is needed to increase our understanding of ODSD and its best therapeutic techniques, including bigger cohort studies with extensive genetic analysis and histopathological examinations.

References

1. Wettasinghe KT et al. A case series of five Sri Lankan patients with ovotesticular disorder of sex development. Clin Pediatr Endocrinol. 2012;21(4):6-73.
2. Bhansali A et al. True hermaphroditism: clinical profile and management of six patients from North India. J Obstet Gynaecol. 2006;26(4):348-50.
3. Hungerford DA et al. The chromosome constitution of a human phenotypic intersex: reconfirmation of a 46-chromosome, xx, apparently non-mosaic “true hermaphrodite.” Hereditas. 1965;52(3):379-86.
4. Meucci R et al. Disorder of sex development: a rare case of a boy with an XY karyotype and magnetic resonance imaging findings of hermaphroditism. JBRA Assist Reprod. 2023;27(2):332-6.
5. Özdemir M et al. Ovotesticular disorder of sex development: an unusual presentation. J Clin Imaging Sci. 2019;9:34.
6. Wang R et al. One case report of ovotesticular disorders of sex development (true hermaphroditism). Chin J Endocr Surg. 2020;6:262-4.
7. Jyoti Chouhan et al. Ovotestis in adolescence: 2 case reports. Urology. 2017;105:171-4.
8. Hughes IA et al. Consensus statement on management of intersex disorders. J Pediatr Urol. 2006;2(3):148-62.
9. Ahmed SF et al. Society for Endocrinology UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development (Revised 2015). Clin Endocrinol (Oxf). 2016;84(5):771-88.
10. Coleman E et al. Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. Int J Transgend. 2012;13(4):165-232.
11. Snodgrass W. Response to commentary to “Results of distal hypospadias repair after pediatric urology fellowship training: a comparison of junior surgeons to their mentor.” J Pediatr Urol. 2016;12(3):164-5.
12. Pasterski V et al. Predictors of posttraumatic stress in parents of children diagnosed with a disorder of sex development. Arch Sex Behav. 2013;43(2):369-75.



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