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15. Hrvatski kongres o **GINEKOLOŠKOJ ENDOKRINOLOGIJI, HUMANOJ REPRODUKCIJI I MENOPAUZI**

8. Kongres Hrvatskog društva **KLINIČKIH EMBRIOLOGA**

s međunarodnim sudjelovanjem

15th Croatian Congress on **GYNECOLOGICAL ENDOCRINOLOGY, HUMAN REPRODUCTION AND MENOPAUSE**

8th Congress of the Croatian Society of **CLINICAL EMBRYOLOGISTS**

with international participation

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Lana Škrgatić

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POZVANA PREDAVANJA/
INVITED LECTURES

1.
PERINATAL MEDICINE MEETS REPRODUCTION

Reccurent pregnancy loss: Evidence based treatment

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Pregnancy loss is defined as the spontaneous loss of pregnancy before the fetus reaches viability. It has been estimated that between 12% to 15% of clinically recognized pregnancies result in spontaneous loss. However, the rate of subclinical pregnancy loss is much greater.

The etiology of RPL can be divided into the following groups: chromosomal, anatomical, thrombophilia, endocrine, autoimmune, infective and inflammatory endometrial, environmental, male factors, and unexplained. Analysis of POC tissue via a molecular-¬based approach should be offered in the RPL setting. Couples who have an identified chromosomal rearrangement should be offered preimplantation genetic testing structural rearrangement (PGT-¬SR) as a treatment option. Anatomical abnormalities of the uterus and/or cervix, congenital or acquired, are associated with RPL. The evidence that resection of the uterine septum reduces recurrent pregnancy loss is uncertain. Evidence for surgical correction of other uterine anomalies is poor. Removal of an endometrial polyp may improve clinical pregnancy rate. There is insufficient evidence for surgical management of intramural fibroids to be used as first-line treatment to improve fertility. Hysteroscopic myomectomy of submucosal fibroids appears to improve pregnancy outcomes. Medical management is not recommended when prompt fertility is desired. Hysteroscopic lysis of adhesions is recommended in cases of intrauterine adhesions. A prospective studies on women with inherited thrombophilia (including FVL, PGM, protein C and S deficiency, antithrombin and MTHFR) failed to demonstrate any difference in LBRs with the use of LMWH or aspirin treatment compared to an untreated control group. In women with antiphospholipid antibodies and RPL (>two pregnancy losses) the greatest efficacy for reducing pregnancy loss was associated with combined antithrombotic therapy (aspirin plus heparin).

Maternal endocrine disorders should be corrected before pregnancy because they are associated with better perinatal outcome

Prospective and randomized trials on immunotherapies such as IV Ig, corticosteroids, intralipid therapy and G-C¬ SF are limited, and results remain conflicting. Given the lack of a clear association between adenomyosis and RPL, there are currently no studies addressing the treatment of adenomyosis in the RPL population. For lifestyle potential risks we should encourage couples to adhere to general health recommendations; eliminating modifiable risk factors such as smoking cessation and alcohol avoidance; decreasing caffeine intake; and reducing unnecessary exposure to heavy metals, plastics and chemicals.

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Recurrent Pregancy Loss: Empiric Management

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Recurrent pregnancy loss (RPL) remains a challenging condition with limited high-quality evidence guiding treatment, leading to reliance on observational data and clinical experience. Despite this, the overall prognosis is favourable, with live birth rates after evaluation around 71–77%. Emotional support is essential, as it improves patient well-being and may enhance outcomes.

When an underlying cause is identified, therapy targets that etiology. Genetic counselling is recommended for couples with chromosomal abnormalities, discussing risks and options including prenatal diagnosis and IVF with preimplantation genetic testing (PGT), which can reduce recurrence, especially in chromosomal translocation carriers. Surgical correction of uterine anomalies such as septa or adhesions improves outcomes, though randomized trials are lacking. Management of medical disorders like antiphospholipid syndrome, thyroid dysfunction, and hyperprolactinemia is advised based on evidence.

In approximately half of cases, RPL is unexplained. For these patients, treatment must balance benefits with risks. Lifestyle modifications to reduce tobacco, alcohol, caffeine intake, and optimize BMI are encouraged despite limited trial data. Controlled ovarian stimulation with human menopausal gonadotropin may aid some women.

Empirical Therapies for Recurrent Pregnancy Loss included progesterone supplementation, antithrombotic therapy and corticosteroids.

Routine supplementation with vaginal micronized progesterone does not statistically significantly improve live birth rates in women with unexplained RPL. Notably, the PRISM and PROMISE trials found no overall benefit for progesterone in this population; however, secondary analyses indicate a possible modest benefit in a subset of women with both prior miscarriage and active early pregnancy bleeding. Current guidelines (e.g., ESHRE 2023, NICE NG126) acknowledge this nuance but do not support universal progesterone use for all unexplained RPL cases. The empirical popularity in practice often reflects provider and patient desire to “do something” when facing repeated loss.

Antithrombotic therapy—whether with low-dose aspirin, low-molecular-weight heparin (LMWH), or both—has not been shown to increase live birth rates in women with unexplained RPL and no antiphospholipid syndrome. Their use is evidence-based only in antiphospholipid syndrome, where efficacy is well established; for other etiologies, these agents remain widely prescribed “just in case” given favourable safety profiles and the lack of better alternatives, but high-quality trials do not support their benefit in the broader RPL population.

The scientific consensus is that corticosteroids do not reduce miscarriage rates in women with unexplained RPL, including those with various immunologic abnormalities. A 2022 Cochrane review and the ESHRE 2023 RPL guideline highlight not only the lack of benefit in live birth but also increased maternal risks—such as gestational diabetes, hypertension, and infection. Corticosteroid therapy is thus currently recommended only within controlled research settings.

In conclusion, management of RPL should prioritize identification and treatment of known causes, provide emotional support, and offer evidence-based interventions for unexplained RPL. Many commonly used therapies lack robust support, and their use should be cautious or restricted to research settings.

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When TSH and Antibodies Are Elevated but Symptoms Are Mild – What Should the Gynecologist Do?

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Subclinical hypothyroidism (SCH) is defined by elevated TSH values above the reference range (most often > 4.0–4.5 mIU/L) with normal free thyroxine (fT4). Positivity for thyroid peroxidase antibodies (TPOAb) is often present but is not required for diagnosis. Although symptoms are not prominent, SCH in women of reproductive age is associated with ovulatory dysfunction, reduced implantation rates, increased risk of miscarriage, preeclampsia, and preterm birth. More recent studies also show that some patients with SCH develop subjective complaints such as chronic fatigue, diffuse hair loss, and mild depression, which affect quality of life even though they do not meet the criteria for overt disease.

According to the American Thyroid Association (ATA 2025) guidelines, routine testing of all women is not recommended; it is indicated in those with infertility, recurrent miscarriages, autoimmune diseases, or a family history of thyroid disease. In preconception care, the goal is to maintain TSH < 2.5 mIU/L. Levothyroxine is recommended for TSH values above the reference range if additional risk factors are present (TPOAb positivity, infertility, previous miscarriages). If SCH is detected in the first trimester, levothyroxine treatment may be considered, while in the second and third trimesters treatment is initiated depending on symptoms and individual patient characteristics. New evidence also highlights a high rate of spontaneous TSH normalization during pregnancy, which is why follow-up testing within three weeks is recommended for borderline results before initiating therapy.

Along with standard treatment, in the preconception period and in women with symptoms but without indication for levothyroxine, the role of supplements such as myo-inositol and selenium is being considered. Clinical studies show that a combination of myo-inositol (600 mg/day) and selenium (83 µg/day) can reduce TSH by about 0.8–1.2 mIU/L, lower TPOAb titers by approximately 20%, and improve subjective complaints, while slowing disease progression. Although these interventions are not part of official guidelines, they represent a promising support in selected patients with mild thyroid dysfunction and symptoms, particularly in the phase of pregnancy planning.

For the gynecologist, it is crucial to promptly recognize subclinical hypothyroidism, individually assess the risks and indications for levothyroxine, and recognize the possibilities of adjuvant measures such as myo-inositol and selenium in order to optimize fertility, pregnancy, and the quality of life of patients.

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From cell to conception: the role of L-carnitine and myo-inositol

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Infertility remains a global health concern, with male and female factors contributing equally to its prevalence. Gamete quality is a key determinant of reproductive success.

Regulation of mitochondrial energy metabolism is essential for oocyte maturation and competence. Oocytes contain a high number of mitochondria and require efficient β-oxidation of long-chain fatty acids, a process dependent on L-carnitine. Mitochondrial dysfunction, aging, poor nutrition, and metabolic disorders can impair meiotic progression, induce oxidative stress, and increase the risk of aneuploidy. Myo-inositol plays a role in phospholipid synthesis, insulin signaling, and FSH signaling. It improves insulin resistance and supports ovulation. Both L-carnitine and myo-inositol may contribute to oocyte quality and reproductive outcomes by enhancing mitochondrial energy production, reducing oxidative damage, and modulating hormonal and metabolic pathways.

Excessive production of reactive oxygen species (ROS), arising from various etiologies, is a major factor in stress-induced sperm dysfunction. In infertile men, DNA fragmentation, mitochondrial dysfunction, and chromosomal aneuploidy occur more frequently. Mitochondria are crucial for sperm energy production through oxidative phosphorylation, and their proper function directly influences sperm motility. Several micronutrients have demonstrated clinical effectiveness in improving sperm parameters. L-carnitine and acetyl-L-carnitine are essential for mitochondrial β- oxidation and ATP synthesis, correlating positively with sperm count, progressive motility and sperm DNA defragmentation.

Evidence from several studies suggests that supplementation with these compounds may contribute to improved sperm motility, oocyte quality, and overall reproductive outcomes.

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Hyperemesis in pregnancy-New Insighsts into Causes and Treatment Options

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Nausea with or without vomiting affects up to 90% of pregnancies, most often in the first trimester. While typically self-limited, symptoms can impair quality of life, daily functioning, and mental health. The severe form, hyperemesis gravidarum, is defined by >5% weight loss, dehydration, electrolyte imbalance, and ketonuria. It can lead to hospitalization, nutritional deficiencies, and rarely, serious complications.

The pathogenesis is not fully understood, but it is known to be multifactorial. Recent studies reveal a significant role of the GDF15 and IGFBP7 genes which are involved in placentation, regulation of appetite and body weight and elevated levels are associated with more severe symptoms. Furthermore, increased hCG levels in the first trimester are closely associated with the onset of symptoms, especially in multiple and molar pregnancies, along with estrogen and progesterone which further slow gastrointestinal motility. Some patients show delayed or dysrhythmic gastric emptying, with worsening of reflux disease. Meta-analyses show that H. pylori infection can increase the risk of hyperemesis gravidarum threefold. Nutritional deficiencies, changes in lipid metabolism, as well as autonomic and psychological factors may also play a role.

The first approach always includes non-pharmacological measures: small, frequent meals with an emphasis on protein, avoidance of fatty, spicy, and strong-smelling foods, drinking fluids between meals in small sips, avoidance of known triggers (strong odors, stuffy rooms, lack of sleep).

If non-pharmacological measures are insufficient, a stepwise pharmacological approach is applied.

1) First line
    a. Pyridoxine (vitamin B6).
    b. If ineffective: doxylamine + pyridoxine
2) Second line
    a. Antihistamines: diphenhydramine, dimenhydrinate.
3) Third line
    a. Dopamine antagonists: metoclopramide, promethazine, prochlorperazine.
4) Fourth line
    a. Serotonin antagonists: ondansetron.
5) Refractory cases
    a) Corticosteroids
    b) Gabapentin, mirtazapine, or olanzapin

Hospitalization is required when the pregnant woman cannot tolerate food and fluids for more than 12 hours, when there are signs of hypovolemia, electrolyte imbalance, or ketonuria.

In hospital, treatment includes:

a) intravenous rehydration (most often Ringer's lactate),
b) correction of potassium, sodium, magnesium,

c) thiamine before glucose to prevent Wernicke's encephalopathy,
d) parenteral multivitamins, folic acid, and vitamin B6.
e) antiemetics

If symptoms persist, enteral nutrition via nasogastric or nasojejunal tube is attempted. Parenteral nutrition is reserved for the most severe, refractory cases due to the risk of sepsis and thrombosis. Most women recover by 20 weeks of gestation.

The simplest and most effective preventive measure is taking multivitamins with folic acid at least one month before conception, which reduces both the risk and severity of symptoms.

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Should detailed ultrasound fetal scan remain the axis of prenatal screening

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Implementation of the molecular screening tests for fetal aneuploidies based on detection of cell free fetal DNA have changed clinical practice. Due to their accuracy and availability, they gained high popularity among clinicians and patients. This practice often led to misconception that molecular tests were certain guarantee of fetal health putting detailed ultrasound scan od fetal anatomy as an adjuvant method. On the other hand, many pregnancies with detected anomalous early fetal development were unreasonably sent to cell free fetal DNA tests. In this debate I will try to discuss the state of the art on this topic and emphasize the outmost importance of early ultrasound screening of fetal anatomy implemented in proper and protocolar clinical practice.

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Redefining ultrasound with new parameters and integration of genetic data

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While the availability and complexity of genetic testing expands, limited information exists regarding obstetrician-gynecologist knowledge of genetics and confidence in providing genetic services. There has been a dramatic change in genetic analysis techniques in prenatal diagnosis in recent years. Fetal DNA in maternal circulation already gained traction in private healthcare, has nowdays, in some countries, been incorporated into public health in high-risk pregnancies where the pregnant woman wishes to avoid an invasive procedure.

Chromosomal microarrays, initially introduced for some specific indications, is replacing karyotyping in most cases. To detect the etiology of monogenic diseases, exon sequencing is now available as gene panels, clinical exome or whole exome sequencing.

Cell-free DNA has emerged as the best screening method due to its high detection rate and low false-positive rate. Expansion of detectable anomalies through cell-free DNA to sex chromosomes, all chromosomes, microdeletions or even selected monogenic anomalies remains controversial.

Fetal ultrasound, which is steadily improving in resolution and precision, offers a possibility of a genetic sonogram in specific indications.

The number of invasive diagnostic procedures has decreased, but they still remain essential for conducting genetic diagnoses. QF PCR is used for rapid diagnosis of common aneuploidies and chromosomal microarray which analyzes the entire genome is now used for diagnosing chromosomal and submicroscopic anomalies. Gene panels and clinical exomes are being introduced for selected fetal malformations following a normal microarray result. Prenatal diagnosis of monogenic diseases is still reserved for gamete donors in assisted reproduction.

Results that are difficult to interpret, such as placental- confined anomalies, mosaicisms, sex chromosome aneuploidies and monogenic diseases are currently the area of prenatal genetics with the most uncertainty.

Genetic counseling in the case of a sex chromosome aneuploidy with a normal ultrasound is already complex, the current abundance of monogenic syndromes makes genetic counseling even more challenging, both, to explain the limitations of the tests and to interpret the results. These are areas of knowledge that many obstetritians currently lack.

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Rheumatic diseases and pregnancy

Velena Radošević[1]

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Rheumatic diseases frequently affect women of reproductive age and may significantly influence pregnancy outcomes. Among them, rheumatoid arthritis (RA) requires particular attention due to its chronic course and need for long-term immunosuppressive therapy. Disease activity often improves during pregnancy but frequently flares postpartum, with potential risks including preeclampsia, intrauterine growth restriction, preterm birth, and low birth weight. Optimal outcomes rely on achieving remission before conception, careful adjustment of therapy, and multidisciplinary care. Safe treatment options during pregnancy include glucocorticoids, hydroxychloroquine, sulfasalazine, azathioprine, and selected biologics—particularly certolizumab pegol, which lacks placental transfer. Contraindicated agents include methotrexate, leflunomide, cyclophosphamide, and mycophenolate. Postpartum relapse is common, but several therapies are compatible with breastfeeding. Certolizumab pegol emerges as a biologic of choice in pregnancy, ensuring both maternal disease control and fetal safety.

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Hyaluronic Acid in Female Genital Health

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Hyaluronic acid (hyaluronan) is a disacharide polymer composed of D-glucuronic acid and N-acetyl-D-glucosamine. It is used in gynecology and obstetrics as a topical reparative medicine and lubricant in the form of vaginal globules or gel. As a component ofthe extracellular matrix, it plays a significant role in angiogenesis, granulation process, tissue regeneration and anti-inflammatory response. With the moist healing mechanism, it promotes non-scarring repair of damaged tissue, most often the mucous membrane, in the following indications: after various forms of vulvovaginal surgery, conization, episiotomy, treatment of perineal tears of all degrees, after vulvovaginal radiotherapy, in postmenopausal vulvovaginal atrophy, other forms of vaginal atrophy / dystrophy, dyspareunia and apareunia, chronic vulvovaginitis, contact dermatitis, wounds on the abdominal wall especially after surgery. Topical hyaluronates have a prolonged effect through slow release and gradual but good absorption into the tissues. They are applied topically 2-3 times a week for a month, and more often.

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Pregnancy outcome at extremely advanced maternal age

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Objectives

Childbearing in the later reproductive years has become increasingly common in high-income countries with potential implications for clinical practice.

Materials and methods

Single-center medical records-based retrospective study, collected between January 2017 and December 2024 at Dept of Ob/Gyn, University Hospital Centre Zagreb, Zagreb were reviewed. We compared the pregnancy course and perinatal outcome of pregnant women of maternal age 44 years with singleton delivery and beyond 24 weeks of gestation (n=168) in 1:2 ratio with those women age 20-29 years (n= 236)

Results

The proportion of pregnant women >44 years increased by 31% in the analysed period. Our research highlighted perinatal risk factors and complications concerning this specific group of patients. Among advanced age pregnant women, preexisting comorbidities burden perinatal outcome. Efforts to reduce unfavourable outcome during pregnancy should target interventions to patient preexisting comorbidity.

Conclusion

Although women in advanced and very advanced maternal age are exposed to greater pregnancy risk, most can achieve a successful pregnancy outcome. Best outcomes appear to be linked to preexisting maternal health and pregnancy care at tertiary level centres.

Long-term implications of late pregnancy will be of special interest in future research.

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POZVANA PREDAVANJA/
INVITED LECTURES

2.
MUST-KNOW TOPICS FOR CLINICIANS AND EMBRYOLOGISTS IN REPRODUCTIVE MEDICINE

How ESHRE certification and accreditation can help raise standarde of reproductive medicine in the region

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During last 2 decades ESHRE try to make standardization in education and training professionals involved in ART, as well as to develop criteria for good clinical practice, and safety and quality in ART process. It was performed with idea to provide equalization of education and practice in Europe, defining minimal standards and terms, with final outcome in improved professional competence and adequate patients care. Certification part of ESHRE activities developed 4 individual certification (embryology certification-exam for clinical and senior embryologist, European Fellow of Reproductive Medicine- exam for subspecialist in Reproductive Medicine, nurses and midwifes certification, ECRES – certification for reproductive surgeons) and 3 institutions certification like ART Centre for good clinical practice, Reproductive medicine subspeciality training and Embryology training. For individual certification education should be according ESHRE curriculum, defined to cover all aspects of the field, and practical skills based on training according to logbook, with sufficient number of interventions to provide trainee competence. Some of the programs will merge in the future, like embryology training and embryology exam, as well as reproductive medicine training and EFRM exam, giving one holistic approach to professional development of embryologist and reproductive medicine clinicians, from beginning of education until end of practice-CPD.

To apply for certification and accreditation individuals and centers should fulfil some terms, very well explained and defined on ESHRE webpage, but whole process is relatively simple. The meaning of certification and accreditation is checking of competency, skills and knowledge by individuals, and for institutions level of organizations and capability to provide adequate service to patients or education to professionals. Certification – accreditation exams motivate individuals for better education and professional development, on the other hand institutions try to improve existing programs and activities, all resulting in better services and patients care. In case of institutional education accreditation, its means first that center fulfilled all condition to be visited, then visit of 2 experts from field with task to detect aberration from program, trainee exposure to work and daily practice, tutorial system and trainee possibilities for learning and practice. From experienced assessors, tutors and center leaders will be informed about week and strong points of educational center, then about possibilities to improve to learning process and visit result. All visits are not performed with idea to critici's center, vice versa, the intention is to help in improving educational capabilities and capacities. Visiting ART center demand from center and from inspector's detail checking point by point for each part of ART process on clinical and laboratory side, as well check in all KPI, what is per se good tool to improve work conditions and by it results too.

All in all, certification process is the way to check and improve our knowledge, organizational skills and professional competence resulting in better care of patients and improved treatment results. This is also process leading to constant institutional and individual improvement.

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https://www.eshre.eu/Accreditation-and-Certification

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IVF in Europe – legislation, regulation, funding and registries in European countries

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ART treatments are permitted in all European countries apart from a few very small ones. However, in practice, the use of the different techniques and the definition of who are allowed accessing them is quite diverse, due to cultural, societal and religious perspectives, and political decisions. Political options about the reimbursement provided to patients are also very distinct from country to country, and sometimes within the same country.

The presentation will focus on the situation of the different European countries regarding the most relevant domains of this complex puzzle that in some places results in important limitations for populations needing ART to pursuit their parental projects, besides promoting very intense cross-border reproductive care movements. Legal framework (techniques permitted, definition of beneficiaries of those techniques, third-party donations boundaries, etc), reimbursement systems (direct and indirect public financial support) and available registries in the different countries will be addressed using the information gathered in a Survey answered by the members of the EIM consortium of ESHRE in 2023. Some information regarding a pan-European registry system (EuMAR) that is being built to improve the quality of the registries and their usefulness to patients, professional communities and policymakers will also be shared

Finally, the last version of the European Atlas of Fertility Treatment Policies organized by Fertility Europe, the European patient's association, will be presented.

Key reference:

European IVF-Monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE); Calhaz-Jorge C, Smeenk J, Wyns C, De Neubourg D, Baldani DP, Bergh C, Cuevas-Saiz I, De Geyter C, Kupka MS, Rezabek K, Tandler-Schneider A, Goossens V. Survey on ART and IUI: legislation, regulation, funding, and registries in European countries-an update. Hum Reprod. 2024 Sep 1;39(9):1909-1924. doi: 10.1093/humrep/deae163. PMID: 39043375; PMCID: PMC11373472.

European and Global ART monitoring & EuMAR

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Registries in ART, whether centre-based, country-based or international have common goals aiming at improving effectiveness and safety of infertility care. Collection of high-quality real-world evidence of day-to-day clinical care on large cohorts during extended periods of observation is crucial for surveillance and biovigilance, including the detection of rare adverse events or reactions. Registry data have the potential for benchmarking towards higher performance and risk reduction or prevention, analysing trends to assess the impact of changes in treatment or policies, and tracking the implementation of new technologies. Data collection systems are highly variable across Europe, challenging the quality of the data. Cycle-level data where each treatment cycle is recorded individually and prospectively, and where standardized health information and a validation process for coverage, completeness and accuracy of the variables is included, is the ideal scenario. In Europe, the largest ART registry was established in 1999 by the European IVF monitoring (EIM) consortium of the European Society of Human Reproduction and Embryology (ESHRE). Over the years, increased participation was observed, and new treatment modalities and techniques were added. However, its main limitation is that only aggregated data from a fragmented legislative and socio-economical landscape are retrospectively collected, presenting the risk of selective reporting and precluding cumulative data analysis at a patient's level. Aiming at a unified approach of medically assisted reproduction (MAR) data collection over Europe, the EuMAR project, co-funded by the EU4Health program and ESHRE, was launched. Its general objective is to move the retrospective aggregated data collection to a prospective cycle-by-cycle, standardized web-based registry, facilitating data sharing for open science across institutes and allowing longitudinal and cross-border follow-up of European MAR data. To this end, new tools were included in the IT solution to preserve the patients' anonymity and allow the calculation of cumulative outcomes across institutions and country borders. An individual reproductive care code allows the linkage of cycles from a same patient or couple, and a clinic swich code is used to maintain the link in cases where a patient moves from one clinic to another. Following a pilot study with 4 countries with different data flow models, stakeholders 'surveys showed that 88% of patients would be in favour of sharing their data and 76% would have more trust in a clinic participating in EuMAR. Moreover, 62% of participating professionals were positive for integrating EuMAR tools in their daily practice and considered it beneficial for improving patients care. Next efforts will be directed towards the expansion of participating pilot countries, the development of means to facilitate onboarding and the establishment of a transition plan between EIM and EuMAR.

Key references:

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European IVF Monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE); Calhaz-Jorge C, Smeenk J, Wyns C, De Neubourg D, Baldani DP, Bergh C, Cuevas-Saiz I, De Geyter C, Kupka MS, Rezabek K, Tandler-Schneider A, Goossens V. Survey on ART and IUI: legislation, regulation, funding, and registries in European countries—an update. Hum Reprod. 2024;39(9):1909-24.

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POZVANA PREDAVANJA/
INVITED LECTURES

3.
WHAT YOU MUST KNOW BEFORE IT'S TOO LATE

Values of HPV Vaccination in Adults

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Human papillomavirus (HPV) is a necessary etiologic factor in cervical cancer and a significant contributor to vulvar, vaginal and anal malignancies. Large epidemiological analyses have demonstrated that population level vaccination programs have substantially reduced the incidence of high grade cervical lesions and generated herd protection effects even in unvaccinated cohorts. Nevertheless, cross sectional data from the U.S. National Health and Nutrition Examination Survey (NHANES) show that a considerable proportion of adult women remain susceptible to infection, with risk factors including younger age at sexual debut, multiple partners, and smoking independently associated with genital HPV prevalence.

In this context, extension of vaccination to adult women has become a critical question. A pivotal open label phase 3 trial demonstrated that the 9 valent HPV vaccine induces robust immunogenicity and maintains a favorable safety profile in women aged 27–45 years, with neutralizing antibody responses comparable to those in the 16–26 year cohort. Furthermore, long term follow up of the FUTURE III study showed significant protection against persistent infection and disease endpoints in women 24–45 years of age, highlighting the clinical relevance of catch up vaccination beyond adolescence.

Collectively, these data indicate that while adolescent vaccination remains the cornerstone of primary prevention, substantial benefit can be achieved by expanding vaccination to adults at risk. High immunogenicity, durable protection, and safety of the 9 valent vaccine support catch up strategies, particularly for women up to 45 years, to further reduce HPV related disease burden at both individual and population levels.

Key references:

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A comprehensive approach to the prevention of HPV-related cancer

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Persistent infection with high-risk types of human papillomavirus (HPV) causes around 530,000 cases of cancer annually worldwide, most commonly affecting women. Cervical cancer is the most frequent localization, while cancers of the anus, vulva, and vagina occur less often, and men are affected in approximately 10% of cases. Despite advances in diagnostics and therapy, cervical cancer remains one of the leading causes of female mortality, particularly in low- and middle-income countries.

Recommendations from professional societies, as well as the goals of the World Health Organization, emphasize the importance of high vaccination coverage against HPV, the implementation of screening using highly specific HPV tests, and the provision of accessible treatment for premalignant and malignant lesions.

Alongside vaccination, which represents one of the key methods of primary prevention, the modern approach to the elimination of cervical cancer also includes screening based on HPV nucleic acid detection. This method has proven to be significantly more effective than cytology and has become the standard in organized screening programs.

The aim of organized and continuous HPV testing is to distinguish between transient and persistent infection, detect the presence of high-risk HPV, and identify cases with possible high-grade premalignant lesions. Available methods include the detection of HPV DNA or mRNA, with tests targeting the expression of oncogenic E6/E7 proteins showing greater sensitivity than those based on the L1 protein.

Evidence shows that HPV test-based screening every five years can reduce cervical cancer mortality by 57%, a finding confirmed by European studies. The recommended age range for screening is 21 to 65 years, with the World Health Organization particularly highlighting women aged 30 to 49 years as the group at greatest risk.

A comprehensive approach to the prevention and treatment of HPV-related cancers includes vaccination, molecular screening, and available therapeutic options, with adaptation of strategies and rational use of resources in less developed settings to achieve greater population coverage.

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The Impact of Obesity on Women's Health from Menarche to Menopause

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Obesity has become one of the leading global public health challenges, with prevalence rising in all age groups. More than half of European women are overweight or obese, and projections indicate further increases in the coming decades. This trend is of particular concern in women's health, as excessive adiposity exerts profound effects on gynecological, reproductive, and metabolic outcomes throughout the life course.

During childhood and adolescence, excess weight accelerates pubertal timing. Overweight girls have up to a two-fold higher risk of early menarche compared with peers of normal weight. Early menarche is associated with increased risks of obesity, type 2 diabetes, cardiovascular disease, and even earlier menopause. In adolescence, obesity disrupts menstrual regularity through insulin resistance and estrogen excess from peripheral aromatization, often manifesting as anovulation, oligomenorrhea, and dysmenorrhea. Obese adolescents are also more likely to develop polycystic ovary syndrome (PCOS), with more severe hyperandrogenism and metabolic dysfunction.

In reproductive years, obesity amplifies hormonal imbalance, resulting in chronic anovulation, heavy bleeding, and unopposed estrogen exposure, which predisposes to endometrial hyperplasia and carcinoma. The majority of women with PCOS are overweight or obese, and adiposity exacerbates both reproductive and metabolic phenotypes. Obesity increases the risk of uterine fibroids, urinary incontinence, and infertility. Women with a BMI $\geq$30 kg/m^2 face a threefold higher risk of infertility, largely due to ovulatory dysfunction, impaired oocyte quality, and reduced implantation potential. Elevated BMI also decreases the success of assisted reproduction and increases miscarriage rates.

Obesity in pregnancy is a major risk factor for adverse maternal and fetal outcomes, including gestational diabetes, hypertensive disorders, preterm birth, macrosomia, and stillbirth. Obese women are more likely to require cesarean section and to experience postpartum complications such as wound infection, hemorrhage, and thromboembolism. Neonatal risks include hypoglycemia, congenital anomalies, and increased need for intensive care. Furthermore, intrauterine exposure to maternal obesity may epigenetically program offspring toward future obesity and metabolic disease, perpetuating an intergenerational cycle of risk.

In perimenopause and postmenopause, cumulative effects of long-term obesity become most evident. Excess adiposity contributes to endometrial cancer, which remains the gynecological malignancy most strongly linked to BMI. Obesity also increases the risk of postmenopausal breast cancer and is associated with poorer cancer outcomes. Beyond malignancies, obese postmenopausal women face elevated risks of cardiovascular disease, diabetes, incontinence, and pelvic floor disorders, as well as diminished quality of life and mobility.

Encouragingly, even modest weight reduction (5–10%) can restore ovulation in anovulatory women, improve fertility outcomes, and reduce obstetric and metabolic complications. Lifestyle modification remains the cornerstone of care; however, the emergence of glucagon-like peptide-1 (GLP-1) receptor agonists has transformed the therapeutic landscape. Agents such as semaglutide and, most notably, the dual GLP-1/GIP receptor agonist tirzepatide, have demonstrated unprecedented efficacy in producing sustained weight loss of >15–20% in clinical trials—outcomes that rival or exceed those of bariatric surgery, but with lower invasiveness and wider applicability. These agents not only improve

cardiometabolic health but may also directly influence reproductive outcomes in women with obesity and anovulatory disorders. Bariatric surgery retains a role for selected patients with severe or refractory obesity, yet pharmacological therapy with GLP-1–based agents is rapidly becoming the preferred option. International guidelines increasingly highlight the importance of integrating such therapies across all stages of a woman's life course, within a multidisciplinary, stigma-free approach focused on long-term health promotion.

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Communicating with People Living with Obesity

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This presentation focuses on effective communication strategies and the importance of empathy when working with patients living with obesity. It emphasizes the crucial aspects of initiating conversations and approaching patients sensitively, recognizing the unique challenges they face. The presentation stresses the significance of treating obesity as a chronic disease that requires long-term management and emphasizes the importance of accurate diagnosis. The "Five A's" approach (Ask, Advise, Assess, Assist, Arrange) will be outlined as a framework for facilitating meaningful discussions about obesity treatment. Understanding the psychological and emotional barriers that patients face is key to providing compassionate care and effectively conveying the importance of addressing obesity.

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Menopause on the Scale: Why do we gain weight more easily during menopause?

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This segment addresses how menopause affects women's body weight and metabolism. The undeniable link between hormonal changes during menopause and obesity is discussed, exploring the various causes of weight gain from psychological to environmental factors. The presentation delves into changes in body composition, reduced insulin sensitivity, and increased cardiovascular risks. Women experiencing overweight or obesity face a higher risk of metabolic complications, cardiovascular disorders, musculoskeletal issues, and cancers. Notably, the link between obesity and gynaecological health in post-reproductive years is highlighted. Common menopause symptoms, such as hot flashes, are examined, with an explanation of the role of adipose tissue and estrogen in modulating these symptoms.

Key references:

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GLP-1 Agonists in the Spotlight: What Do the Clinical Trials Really Tell Us?

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Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) represent a major advance in obesity therapy through their dual effects on appetite regulation and energy balance. Semaglutide 2.4 mg once weekly (Wegovy) is a GLP-1 RA approved for chronic weight management in adults with obesity or overweight with weight-related comorbidities. Its mechanism of action involves slowing gastric emptying, enhancing satiety, and reducing energy intake, thereby achieving clinically significant weight loss.

Semaglutide 2.4 mg has been studied in more than 25,000 people with overweight or obesity across 14 clinical trials, providing a robust evidence base for its efficacy and safety. Within the STEP program, semaglutide demonstrated superior outcomes compared with placebo and other anti-obesity treatments. In STEP 5, semaglutide plus lifestyle intervention produced a mean weight loss of 15.2% over 104 weeks, with concurrent improvements in cardiometabolic risk factors. The STEP UP trial confirmed a dose-dependent effect, with the highest dose achieving approximately 21% weight reduction at 72 weeks. Evidence also suggests cardiovascular risk reduction of up to 20%, underscoring its broader health benefits. An overview of recent real-world studies also highlight how guidelines position obesity treatment. Its proven efficacy and safety profile marks semaglutide as crucial part of obesity management protocols.

Safety outcomes were consistent with the GLP-1 RA class. The most frequent adverse events were mild-to-moderate gastrointestinal symptoms, typically transient during dose escalation. Rates of treatment discontinuation were low (3.3–5.9%), and serious adverse events were comparable or lower than placebo. No increase in pancreatitis was observed.

In summary, semaglutide offers durable, clinically meaningful weight loss with a favorable safety profile, positioning it as a cornerstone therapy in modern obesity management guidelines.

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GLP-1 Agonists – To Whom, When, and for How Long – Case Study

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Introduction

Menopause is a period of profound hormonal and metabolic changes that increases the risk of obesity and its related complications. Obesity is a major risk factor for cardiovascular and metabolic diseases, and it is well established that almost two-thirds of women in menopause die from cardiovascular causes. In this context, early recognition and effective management of obesity are essential for improving quality of life and reducing the risk of chronic non-communicable diseases (NCDs). GLP-1 receptor agonists have emerged as a novel and highly effective therapeutic option that not only promote significant weight reduction but also exert beneficial effects on cardiometabolic health and menopausal symptoms.

Objective

To present a clinical case of a postmenopausal woman with persistent symptoms despite hormone replacement therapy (HRT), in whom the addition of a GLP-1 receptor agonist resulted in marked clinical and metabolic improvements.

Case presentation

A 57-year-old postmenopausal woman (postmenopausal for 2 years) on HRT due to severe vasomotor symptoms and insomnia presented with persistent complaints, obesity (BMI 29,7 kg/m^2) and dyslipidemia. Despite optimization of HRT, both symptoms were less pronounced but still present and laboratory abnormalities remained unchanged. After comprehensive evaluation, semaglutide was initiated as part of an obesity management strategy. Over just a six-month treatment period, the patient achieved a 14% weight reduction, normalization of lipid profile, and complete regression of menopausal symptoms. She is still on both therapies and reported substantial improvement in quality of life, increased energy levels, and restored sleep quality.

Conclusion

Obesity in menopause represents an important but frequently overlooked therapeutic target. Its effective management, particularly using GLP-1 receptor agonists, can significantly reduce the risk of cardiovascular and metabolic diseases while simultaneously improving menopausal quality of life. This case highlights that combining HRT with pharmacological obesity treatment may provide a synergistic benefit, underscoring the importance of an integrated approach in the management of postmenopausal women.

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POZVANA PREDAVANJA/
INVITED LECTURES

4.
MENOPAUSE: IT'S TIME TO STOP IGNORING IT

Perimenopause – the journey that changes everything

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Perimenopause is a transitional life stage marked by fluctuating estrogen and progesterone production, leading to diverse physical, emotional, and cognitive changes. Menstrual cycles become irregular, sometimes shorter, longer, or absent, while hot flashes, night sweats, sleep disturbances, headaches, joint stiffness, and skin or hair changes reflect the systemic influence of hormonal shifts. Because estrogen receptors are widespread, symptoms can affect nearly every organ system, making perimenopause a whole-body experience with wide individual variability.

Emotional lability, irritability, tearfulness, and brain fog are common, yet midlife stressors—career shifts, caregiving, financial pressure—intertwine with biology, shaping personal experience. Fertility declines but persists until menopause; contraception remains necessary unless menopause is confirmed, while those wishing to conceive face reduced chances, sometimes aided by assisted reproduction.

Declining estrogen increases risks for osteoporosis and cardiovascular disease, highlighting the importance of nutrition, exercise, and regular medical care. Adequate protein, calcium, vitamin D, healthy fats, and fiber support metabolic, skeletal, and cardiovascular health. Physical activity combining resistance, aerobic, and mobility training helps preserve strength, bone density, and mental wellbeing. Sleep, often disrupted, is a cornerstone of resilience; consistent routines, relaxation practices, or cognitive-behavioral strategies can improve rest.

Mental health requires equal attention, as fluctuating hormones can exacerbate anxiety or depression, while social silence around perimenopause fosters isolation. Counseling, peer support, and mindfulness can help normalize this stage. Medical management ranges from lifestyle approaches to menopausal hormone therapy (MHT), which can effectively relieve vasomotor symptoms and protect bone in appropriately selected women. Non-hormonal medications and localized treatments offer alternatives for genitourinary or vasomotor complaints, while supplement use requires professional guidance due to variable efficacy.

Ultimately, perimenopause represents a bridge between reproductive and post-reproductive life. With informed choices, lifestyle adjustments, and supportive medical care, it can be navigated not only as a challenge but as an opportunity to strengthen long-term health and wellbeing.

Key references:

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The Importance of Individualizing Menopausal Hormone Therapy and Its Impact on Cardiovascular Health in Postmenopausal Women

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Menopausal hormone therapy (MHT) remains the most effective treatment for moderate-to-severe vasomotor symptoms and the prevention of postmenopausal bone loss. However, evidence from randomized controlled trials and meta-analyses has demonstrated that the cardiovascular (CV) effects of MHT depend critically on the timing of initiation, the patient's individual risk profile, and the choice of regimen. International guidelines, including the 2022 Position Statement from the North American Menopause Society (NAMS) and the 2020 European Menopause and Andropause Society (EMAS) recommendations, emphasize that MHT should be considered within 10 years of the final menstrual period or before the age of 60 in women with low baseline CV risk, as it is then associated with neutral or even favorable cardiovascular outcomes.

Individualized risk assessment is central to safe prescribing. Gynecologists should systematically evaluate personal and family history of cardiovascular disease, measure blood pressure, fasting glucose, lipid profiles, body mass index, and screen for hypertension, dyslipidemia, diabetes, smoking, and metabolic syndrome. Risk stratification tools such as SCORE2 or ASCVD estimators help categorize women into low, intermediate, or high CV risk groups. Women at low CV risk are appropriate candidates for oral or transdermal estrogen combined with a progestogen with a neutral metabolic profile (e.g. micronized progesterone or dydrogesterone). In intermediate risk patients, transdermal estradiol is preferred due to a lower impact on coagulation parameters and triglycerides. In women with established cardiovascular disease or high calculated risk, systemic MHT is generally not recommended; if treatment is necessary for severe symptoms, a minimal effective dose of transdermal estradiol under close cardiology supervision may be considered.

For symptomatic women in whom MHT is contraindicated or deemed unsuitable because of elevated CV risk or a history of hormone dependent malignancy, non hormonal alternatives are now available. Fezolinetant, a selective neurokinin 3 receptor antagonist recently approved for the treatment of vasomotor symptoms, modulates hypothalamic thermoregulatory pathways and has demonstrated significant reductions in hot flash frequency and severity without stimulating the endometrium or breast tissue and without adversely affecting coagulation or lipids.

An individualized approach allows clinicians to balance symptom control with long term safety. Integrating a structured CV risk assessment into decision making ensures that MHT is tailored to each patient's profile, while non hormonal options such as fezolinetant expand the therapeutic armamentarium for those with contraindications. This paradigm supports optimized quality of life while maintaining cardiovascular safety in postmenopausal women.

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Fezolinetant: A Novel Non-Hormonal Option for Vasomotor Symptoms in Menopause

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Vasomotor symptoms (VMS), including hot flushes and night sweats, affect up to 80% of women during the menopausal transition, with moderate-to-severe symptoms in as many as 40%. Once considered benign and transient, VMS are now recognized as markers of broader health implications. Persistent VMS are linked to impaired sleep, reduced quality of life, mood disturbances, and occupational difficulties, while epidemiological studies associate frequent or severe VMS with elevated risks of cardiovascular disease, diabetes, osteoporosis, cognitive decline, and dementia. These findings underscore the need for effective, safe, and acceptable treatment options beyond traditional hormonal therapy.

Menopausal hormone therapy (MHT) remains the most effective intervention, reducing symptoms by up to 80%. However, contraindications such as estrogen-sensitive malignancies, liver disease, or thromboembolic risk, together with persistent patient hesitancy, limit its use. Until recently, non-hormonal pharmacologic alternatives were restricted to SSRIs, SNRIs, and gabapentinoids, which offer only moderate efficacy and carry tolerability concerns. This therapeutic gap has catalyzed the search for targeted non-hormonal agents.

Advances in neuroendocrine science have identified hypothalamic KNDy neurons—co-expressing kisspeptin, neurokinin B (NKB), and dynorphin—as key regulators of thermoregulation. Declining estrogen removes inhibitory control over these neurons, resulting in hyperactive NKB signaling, narrowed thermoneutral zone, and vasomotor instability. Fezolinetant, a selective neurokinin-3 receptor (NK3R) antagonist, directly inhibits NKB signaling to restore thermoregulatory balance. Administered orally once daily at a standardized 45 mg dose without need for titration, fezolinetant represents a first-in-class, mechanism-based non-hormonal therapy.

Phase III trials have confirmed its efficacy and safety. In SKYLIGHT 1 and SKYLIGHT 2, fezolinetant reduced VMS frequency and severity significantly versus placebo, with improvements observed by day 3 and sustained through week 12. More than 50% of women achieved ≥50% reduction in symptoms, alongside marked improvements in sleep quality and menopause-specific quality-of-life scores. The SKYLIGHT 4 trial established long-term safety, showing no endometrial stimulation and only rare, transient elevations in liver enzymes. The DAYLIGHT phase 3b trial extended evidence to women unsuitable for MHT, again demonstrating significant reductions in VMS frequency and intensity and improved sleep outcomes. A 2024 network meta-analysis positioned fezolinetant as the most effective and best tolerated non-hormonal agent, outperforming SSRIs, SNRIs, and gabapentin.

In conclusion, fezolinetant represents a paradigm shift in the management of VMS, expanding the therapeutic armamentarium for women unable or unwilling to use hormones. With its rapid onset, robust efficacy, and generally favorable safety profile, fezolinetant is poised to become the cornerstone of personalized, hormone-free menopause care.

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Fezolinetant – To Whom, When, and for How Long – Case Study

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Introduction

Vasomotor symptoms (VMS), particularly hot flushes, are among the most common and burdensome complaints of menopausal women, significantly impairing quality of life, sleep, mood, and daily functioning. Hormone replacement therapy (HRT) remains the gold standard treatment, but many women either have contraindications or decline hormones due to fear of adverse effects, especially those related to breast cancer and cardiovascular risk. The unmet need for effective non-hormonal therapies has been a longstanding challenge in gynecological endocrinology. Fezolinetant, a selective neurokinin-3 receptor (NK3R) antagonist, represents a novel and targeted non-hormonal approach to treating moderate to severe VMS. By modulating hypothalamic thermoregulatory dysfunction thought to drive hot flushes, fezolinetant offers a new option for women who cannot or will not use HRT.

Objective:

To present a case of a postmenopausal woman with bothersome vasomotor symptoms who refused HRT due to fear of hormonal risks, and in whom treatment with fezolinetant provided significant symptom relief and improvement in quality of life.

Case presentation:

A 53-year-old postmenopausal woman presented with frequent, severe hot flushes (10–12 per day) and night sweats leading to fragmented sleep and daytime fatigue. Despite counseling on the benefits and safety profile of modern HRT, she declined hormonal therapy, citing fear of breast cancer due to strong family history. After evaluation excluded secondary causes, fezolinetant 45 mg daily was initiated. Within four weeks, she reported a 70% reduction in frequency and severity of hot flushes, improved sleep continuity, and marked relief of fatigue. By 12 weeks, hot flushes had decreased to one to two per day, sleep quality normalized, and mood and concentration improved. Laboratory monitoring (including liver enzymes) remained within normal limits. The patient expressed high satisfaction with therapy and chose to continue treatment.

Conclusion:

Fezolinetant provides an effective, safe, and well-tolerated non-hormonal alternative for the treatment of vasomotor symptoms in postmenopausal women, particularly those unwilling or unable to use HRT. This case illustrates how fezolinetant can bridge an important therapeutic gap, offering meaningful relief and improved quality of life. While the optimal duration of therapy remains to be fully defined, current evidence supports its use for sustained symptom control. Incorporating fezolinetant into clinical practice broadens the spectrum of individualized care for women in menopause.

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Efficacy and safety of fezolinetant for the treatment of moderate to severe vasomotor symptoms associated with menopause in women considered unsuitable for hormone therapy: the phase 3b DAYLIGHT study

Jandric I.[1] (presenting on behalf of authors):

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Aim

Hormone therapy (HT) is an effective treatment for vasomotor symptoms (VMS) associated with menopause. However, it is not always appropriate and many women do not take HT. Therefore, well tolerated and effective nonhormonal therapies for VMS are desirable. Fezolinetant is approved as a treatment option for moderate to severe VMS in many regions, including North America, Europe, Asia, and Australia at a dose of 45 mg once daily. The objective was to assess the efficacy and safety of fezolinetant for the treatment of moderate to severe VMS associated with menopause in women considered unsuitable for HT.

Methods

DAYLIGHT was a phase 3b, randomised, double-blind, 24-week study (NCT05033886). The women were randomised to placebo or fezolinetant 45 (1:1) once daily and VMS were recorded daily using an electronic diary. Primary and key secondary efficacy endpoints were analysed using mixed model for repeated measures. Women aged ≥40 to ≤65 years with moderate to severe VMS who were unsuitable for HT based on: contraindications, caution (prior medical history), stoppers (lack of efficacy, side effects, or medical advice), or averse (made informed choice not to take HT after discussion with clinician). The primary endpoint was mean change in daily VMS frequency of moderate to severe

episodes from baseline to Week 24. Mean change in VMS severity (key secondary endpoint) and safety were also assessed.

Results

Overall, 452 women received at least one dose of study drug (placebo n=226; fezolinetant n=226), including HT contraindicated (50, 11%), caution (165, 37%), stoppers (69, 15%), and averse (168, 37%). At 24 weeks, fezolinetant 45 mg significantly reduced VMS frequency (least squares [LS] mean difference: –1.93; 95% confidence interval [CI] –2.64, –1.22; p<0.001) and VMS severity (LS: –0.39; 95% CI –0.57, –0.21; p<0.001) vs placebo. Improvements vs placebo were observed as early as week 1. Similar incidences of treatment-emergent adverse events (TEAEs; placebo: 61.1%, fezolinetant: 65.0%) and serious TEAEs (3.5%, 4.4%) were observed in both groups. Most common fezolinetant group TEAEs: COVID-19 (13.3%), headache (8.8%), and fatigue (5.8%).

Conclusions

The phase 3b DAYLIGHT study showed that fezolinetant 45 mg was efficacious and well tolerated for moderate to severe VMS in women considered unsuitable for HT. These results highlight the utility of fezolinetant as an effective nonhormonal treatment for those who cannot or choose not to receive HT.

Disclosures

Ivan Jandric: participated on advisory boards for Astellas Pharma Inc., had received travel support from Astellas, had received honoraria for lectures from Gedeon Richter, Board Member of the Croatian Menopause Society.

Katrin Schaudig: consultant and lecturer for aidhere, Astellas, Bayer HealthCare, Besins Healthcare, Exeltis, Gedeon Richter, Jenapharm, Mylan, Novo Nordisk, Organon, Theramex, Viatris, Laborarztpraxis Rhein-Main; President of the German Menopause Society (Deutsche Menopause Gesellschaft e.V.).

Xuegong Wang, Kentaro Miyazaki: employee of Astellas Pharma Inc.

Céline Bouchard: has received research grants from Astellas, Exeltis, Incyte, and Mylan Technologies; has received honoraria from Astellas, BioSyent, Lupin Pharma, and Pfizer; has received travel support from Bayer HealthCare and Searchlight; and has participated on advisory boards for Astellas, Bayer HealthCare, and Lupin Pharma; Vice-President of the Canadian Menopause Society.

Angelica Lindén Hirschberg: consultant for Astellas, and has received honaria from Exeltis, Bayer Healthcare and Gedeon Richter, and research grants from Besins Health Care and Avia Pharma.

Antonio Cano: was the past President of the European Menopause and Andropause Society; and is a consultant for Astellas, Theramex, and Viatris.

Marla Shapiro C.M.: has attended advisory boards and/or received consulting fees or honoraria from Aspen, Astellas, Bayer HealthCare, BioSyent, Duchesnay, GSK, Merck, Mithra, Pfizer, Searchlight, Sprout, Sunovion, and Therapeutics MD; and fulfils a leadership or fiduciary role for the International Menopause Society, Research Canada, and Terry Fox Research Institute.

Petra Stute: reports receiving fees/consulting fees from Astellas, Bayer, Besins Healthcare, Effik, Exeltis, Gedeon Richter, Jenapharm, Labatec, Theramex, and Viatris.

Weizhong He, Ludmila Scrine: employee of Astellas Pharma Inc at the time of development.

Rossella E. Nappi: reports past financial relationships (lecturer, member of advisory boards, and/or consultant) with Exeltis, HRA Pharma, Novo Nordisk, and Pfizer; ongoing relationships with Abbott, Astellas, Bayer HealthCare, Besins Healthcare, Biocodex, Fidia, Gedeon Richter, Merck & Co, Shionogi, Theramex, Viatris, and Vichy Laboratories; and serving as President of the International Menopause Society.

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New in the treatment of vaginal atrophy – estriol

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Vaginal atrophy, a key component of genitourinary syndrome of menopause, is characterized by thinning of the vaginal epithelium, loss of elasticity, and increased pH, resulting in symptoms such as dryness, irritation, dyspareunia, and recurrent urinary discomfort. Local estrogen therapy remains the most effective treatment, and recent focus has shifted toward the use of ultra-low-dose estriol.

Estriol, a naturally occurring weak estrogen, provides localized action with minimal systemic absorption, restoring the vaginal mucosa, improving lubrication, and supporting a healthy microbiota. Modern formulations, such as ultra-low-dose gels and vaginal tablets often combined with probiotics, have demonstrated high efficacy and safety, even in sensitive populations, including breast cancer survivors under medical supervision. These approaches minimize systemic hormone exposure and do not generally require progestogen supplementation.

Non-hormonal alternatives, such as hyaluronic acid, may benefit those with contraindications to estrogen, although they are less effective in severe cases. Emerging evidence supports combination strategies, including pelvic floor exercises, to optimize symptom relief and quality of life.

Current recommendations emphasize personalized therapy, prioritizing ultra-low-dose estriol for moderate to severe symptoms, while reserving non-hormonal agents for milder cases or specific clinical scenarios.

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Innovative Therapeutic Approach to Women in Perimenopause and Menopause

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Hot flashes, the most common vasomotor symptom of perimenopause and menopause, are not only a quality-of-life issue but may also serve as a marker of underlying vascular changes. Increasing evidence suggests that frequent or severe hot flashes are associated with endothelial dysfunction and an elevated risk of future cardiovascular disease, highlighting the need for early, safe, and effective symptom management in midlife women.

The thermoregulatory dysfunction underlying hot flashes is closely related to changes in hypothalamic activity, particularly involving KNDy (kisspeptin/neurokinin B/dynorphin) neurons, which are sensitive to estrogen withdrawal. These neurons influence the thermoregulatory hypothalamic zone via serotonergic and noradrenergic pathways. Disruption of this system leads to narrowed thermoregulatory thresholds, triggering sudden heat-dissipating responses such as vasodilation and sweating. This explains the rationale behind therapies targeting serotonin, norepinephrine, or neurokinin receptors in alleviating vasomotor symptoms.

Treatment options include hormone replacement therapy (HRT), which remains the gold standard, as well as non-hormonal agents such as SSRIs/SNRIs, gabapentin, clonidine, and newer neurokinin receptor antagonists. However, concerns regarding side effects and contraindications often limit their use, especially in women with hormone-sensitive conditions.

Serelys, a non-hormonal, plant-derived product based on purified cytoplasmic pollen extracts, emerges as an innovative and well-tolerated option. It contains bioactive compounds that modulate serotonergic activity and exhibit antioxidant effects, contributing to thermoregulatory balance without acting on estrogen receptors. Clinical studies have shown that Serelys significantly reduces the frequency and intensity of hot flashes, improves sleep quality, and enhances overall well-being.

Importantly, Serelys is particularly suitable for women previously treated for hormone receptor-positive breast cancer, where hormone therapy is contraindicated. Preclinical data indicate that Serelys does not promote proliferation in hormone-sensitive breast cancer cell lines, supporting its safety in this high-risk group.

As part of a modern, individualized approach to midlife women's health, Serelys offers an effective and safe alternative for managing vasomotor symptoms, while potentially mitigating associated cardiovascular risks. Continued research will further define its role within comprehensive menopausal care.

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POZVANA PREDAVANJA/
INVITED LECTURES

5.
FROM OUTPATIENT CLINIC TO OPERATING ROOM: ENDOMETRIOSIS AS A CHALLENGE OF MODERN GYNECOLOGY

How understanding the pathogenesis of endometriosis shapes pharmacological therapy

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Endometriosis is a chronic, estrogen-dependent, inflammatory condition affecting approximately 10% of women of reproductive age. It is increasingly recognized as a complex, multifactorial disorder, in which hormonal, immunological, genetic, epigenetic, and environmental factors interact to drive disease initiation and progression.

One important contextual change in reproductive biology is the dramatic increase in the number of menstrual cycles a woman experiences during her lifetime. Historically, due to later menarche, earlier menopause, and frequent pregnancies with prolonged breastfeeding, women experienced only about 50–100 menses in a lifetime. In contrast, women today, with earlier menarche, later menopause, fewer pregnancies, and shorter breastfeeding periods, experience approximately 400–450 menstrual cycles. This exponential increase in menstrual frequency is believed to augment retrograde menstruation, chronic peritoneal inflammation, oxidative stress, and subsequent risk for endometriosis. Thus, endometriosis can be regarded as, at least in part, a “disease of civilization,” emerging from altered reproductive patterns.

Understanding the pathogenesis of endometriosis has significantly influenced medical therapy. Traditional approaches aimed broadly at suppressing estrogen production, most commonly using combined oral contraceptives, progestins, and gonadotropin-releasing hormone (GnRH) agonists. More recently, titratable GnRH antagonists have become available, offering improved tolerability and flexibility. Despite these advances, many women remain symptomatic due to mechanisms such as progesterone resistance, chronic inflammation, angiogenesis, and neurogenesis.

Experimental therapeutic targets have emerged based on these mechanisms. Selective progesterone receptor modulators (SPRMs), such as mifepristone and ulipristal, showed efficacy in reducing pain and lesion size, but safety concerns, particularly hepatotoxicity and endometrial changes, limit their use. Immunomodulatory approaches, such as anti-TNF therapy, JAK inhibitors, and IL-1 antagonists, have shown promise in preclinical models, though human trials have thus far been inconclusive. Similarly, anti-angiogenic strategies (e.g., bevacizumab) and antagonists of neurotrophins (such as anti-NGF antibodies) demonstrated reduction in lesion growth and pain in animal models but remain experimental in humans.

Epigenetic modulation represents another frontier. Aberrant DNA methylation and histone acetylation in endometriotic tissue suggest that DNMT and HDAC inhibitors could restore progesterone sensitivity and suppress proliferation, though clinical application is limited by toxicity. Parallel research into metabolic modulators such as metformin and statins, as well as antioxidants like melatonin and resveratrol, provides further avenues for therapy, with preliminary evidence indicating reduced lesion growth and improved pain outcomes.

Microbiome-targeted strategies are also under investigation. Dysbiosis in the gut and genital tract may contribute to immune dysregulation and estrogen metabolism. Early studies on probiotics, prebiotics, and dietary interventions (such as gluten-free or anti-inflammatory diets) suggest symptomatic benefit in some women, although robust randomized controlled trials are lacking.

Finally, the recognition of a neuropathic component in endometriosis-associated pain has introduced neuromodulators into therapeutic discussions. Off-label use of gabapentin and duloxetine has been

evaluated, though with mixed results, and low-dose naltrexone (LDN) is increasingly discussed as a potential adjunct, given its dual analgesic and immunomodulatory effects.

In summary, understanding the multifactorial pathogenesis of endometriosis has transformed therapy from empirical suppression of estrogen to a more personalized, mechanism-based approach. While current mainstays remain hormonal suppression with contraceptives, progestins, and GnRH analogs/antagonists, emerging therapies targeting inflammation, angiogenesis, neurogenesis, epigenetics, metabolism, and the microbiome represent the future. Tailoring therapy to individual phenotypes, fertility goals, and molecular markers offers the greatest potential for improving outcomes in women with this chronic and often debilitating disease.

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Surgery first, then pregnancy naturally or via IVF

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Previous experiences, institutional recommendations, literature, and guidelines consistently advocate for a fully individualized approach in the treatment of patients with endometriosis. Treatment of endometriosis can be surgical or non-surgical. Although my personal opinion is that endometriosis is a "primarily surgical disease," over time, with experience, and due to the nature of the disease and its unclear etiology, the approach to patients has become significantly more "conservative" in recent years. This is particularly true for infertile patients!

It is a fact that patients with endometriosis, even minimal endometriosis and ovarian endometriomas, often have a reduced ovarian reserve. The anti-Müllerian hormone (AMH) level is frequently low from the outset. Unfortunately, surgeries for endometriosis—such as the removal of ovarian endometriomas or radical pelvic surgeries for deep infiltrating endometriosis (DIE)—can further compromise the likelihood of pregnancy in these patients. Regardless of the presence of DIE, in primarily infertile patients, it is always necessary to weigh the benefits of surgery against the risks it poses in relation to infertility.

Increasing the chances of pregnancy through surgery in patients with endometriosis involves addressing factors that may contribute to reduced fertility. It is clear that we are not discussing direct causes of infertility, as we know of many patients with endometriosis who have spontaneously conceived. This fact obliges us to adopt a more conservative approach to treatment. In the past, we often pointed out suboptimal surgical outcomes in patients with DIE, largely due to the lack of endoscopic surgical skills among many surgeons who would "incidentally" address both endometriosis and infertility. Today, with an increasing number of skilled endoscopic surgeons capable of performing operations according to the principles of retroperitoneal pelvic surgery, we face a situation where the radical nature of these surgeries requires us to highlight risks that may exacerbate the causes of infertility and further compromise the possibility of conception, whether naturally or through in vitro fertilization (IVF).

The goal of surgical treatment is to alleviate disease symptoms, improve patients' quality of life, and increase the likelihood of spontaneous pregnancy or successful IVF. This includes removing ovarian endometriomas, regardless of the method, to enhance ovarian function and accessibility for potential IVF procedures, restoring "more normal" anatomical relationships in the pelvis, addressing symptoms that may interfere with normal intercourse, and, where possible, resecting uterine adenomyosis, which can be an additional cause of infertility.

When considering indications for surgery, the risks must always be kept in mind—primarily the further reduction of ovarian reserve, especially in the presence of endometriomas. Additionally, potential complications, which are often serious and can further impact pregnancy, include urinomas, enterovaginal fistulas, fibrosis, adhesions, and extensive resections of adenomyosis that may worsen the chances of conception.

Situations where primary surgical treatment of endometriosis is warranted include ureteral obstruction by an endometriotic nodule, most commonly at the parametrial level, leading to hydroureter and hydronephrosis. Similarly, bowel obstruction (often circumferential) that may result in ileus also necessitates surgery.

Primary surgical treatment is indicated in patients who cannot engage in normal intercourse due to

pain or other symptoms but have a normal or relatively preserved ovarian reserve. It is also indicated in patients whose quality of life is significantly impaired, rendering them unable to function normally.

Primary surgery is considered in patients whose ovaries are inaccessible or difficult to access for oocyte retrieval during IVF or who show no response or poor response to ovarian stimulation.

If all conservative methods aimed at “suppressing endometriosis” have been exhausted, regardless of the approach, and the patient fails to conceive after multiple IVF attempts, surgery is indicated with the goals outlined above.

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Avoid surgery, go straight to IVF

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Endometriosis is a chronic disease with many debilitating symptoms like dysmenorrhea, dyspareunia, dyschesia, fatigue, pelvic pain, bloating but also infertility. The prevalence rate of endometriosis in infertile women is estimated to be 25–40% and it is responsible for approximately 10% of the indications for in vitro fertilization (IVF).

One of the mechanisms that could explain negative impact of endometrioma on ovarian function is toxic effect it has on healthy tissues around the cyst in form of high levels of cell damage-mediating factors such as proteolytic enzymes, inflammatory mediators, reactive oxygen species (ROS), and iron.

Endometriosis is likely to reduce the quality of oocytes. But, on the other hand, surgical intervention for endometrioma may increase the risk of infertility by reducing the ovarian reserve. Data, mostly uncontrolled, would favor surgery at any stage of endometriosis, increasing the chances of natural conception compared to expectant management, but approach to patients with endometriosis and infertility should be tailored based on different parameters like location of the disease, severity of symptoms, age of the patient.

Management options should include surgery, in-vitro fertilization (IVF) or combination of both. Regarding the outcome of IVF in patients with endometriosis, the reduction in the IVF pregnancy rate was approximately 20% in patients with mild endometriosis, while the reduction was >50% in patients with extensive/severe endometriosis.

Pending question is when to operate on endometriomas and when to skip the surgery. Upon a decision-making we need to consider at least five points: the age of the woman, the presence/absence of pain, the number of previous interventions, ovarian reserve and the possibility of occult malignancy.

In conclusion, although the optimal treatment cannot presently be proposed, there is insufficient evidence to support a strategy of systematic surgical treatment of endometriomas before IVF–ICSI cycles.

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Preservation of Ovarian Reserve in Patients with Ovarian Endometriosis Using the Helica Thermal System (Helica)

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Objective

Surgical treatment of ovarian endometriosis often involves cystectomy, which may lead to a reduction in ovarian reserve due to inadvertent removal of healthy ovarian tissue. In order to preserve fertility potential, less invasive approaches such as ablation have been developed, with helium and laser ablation being among the most notable. The helium system (Helica) enables precise ablation of endometriotic lesions with minimal damage to surrounding tissue. The aim of this study was to evaluate the impact of the Helica system on ovarian reserve and recurrence rate in patients with ovarian endometrioma.

Materials and Methods

A retrospective analysis was conducted on 18 patients with symptomatic unilateral or bilateral ovarian endometriomas measuring ≥5 cm and <10 cm, treated with the helium system between August 2024 and March 2025. All patients were aged 18–40 years, with no prior ovarian surgery and no endocrine disorders. Anti-Müllerian hormone (AMH) levels and antral follicle count (AFC) were assessed preoperatively and at 6 months postoperatively. During surgery, the cyst content was evacuated, the cyst wall surface was vaporized using the helium system, and in selected cases, biopsies were taken for histological evaluation. The recurrence rate was also monitored.

Results

At 6 months postoperatively, a reduction in AMH levels of 3–5% was observed, while AFC remained stable. In patients followed for more than 6 months, AFC showed a slight increase. During the study period, one recurrence was recorded, corresponding to a low recurrence rate (5.6%).

Conclusion

Helium ablation represents an effective and fertility-sparing surgical method for the treatment of ovarian endometriosis, with minimal impact on ovarian reserve and a low recurrence rate. These findings support the use of helium ablation as a safe alternative to standard cystectomy, particularly in women seeking to preserve fertility. Further prospective studies with longer follow-up are warranted to confirm the long-term efficacy of this method

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Endometriosis and the risk of adverse pregnancy outcomes

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While there is a good deal known about the impact of endometriosis on infertility, which is supported with fairly reliable data, the same cannot be said for the data on the impact on the course and outcome of pregnancy.

The data from large cohort studies are most reliable, but meta-analyses may compile too many errors from individual, very heterogeneous studies. It is important for each center that deals with patients with endometriosis to constantly validate its own data, as well as to publish individual interesting cases that show the great diversity of both endometriosis and adenomyosis and help to clarify many uncertainties at the individual level.

A study conducted on a large French cohort sample (368,935 pregnancies between 1999 and 2016) examined the association between endometriosis and selected adverse pregnancy outcomes. Pregnant women diagnosed with endometriosis represented 0.57% of all participants. The highest and most consistent increased risk was for placenta previa. The risk of preterm birth, preeclampsia, and some forms of small for gestational age (SGA) was also partially confirmed. The study warns of the possibility of underestimation of the association due to undiagnosed endometriosis in the control group.

Furthermore, a meta-analysis of 39 cohort studies shows that endometriosis is associated with an increased risk of several pregnancy complications, such as gestational hypertension, preeclampsia, preterm labor, placenta previa, placental abruption, cesarean section, and stillbirth. It can be said that endometriosis significantly increases the risk of several serious pregnancy complications, but due to the heterogeneity of the data, open questions remain that call for further, more carefully designed studies.

The retrospective cohort study performed in Department of Human Reproduction in Ljubljana focused on obstetric outcomes in women who underwent surgery for bowel endometriosis. Seventy-one pregnancies from women who had bowel endometriosis surgery were documented and matched to 213 controls. Women with bowel endometriosis, even after surgery, remain at elevated risk of placenta previa, abnormal labor, emergency cesarean delivery, hemorrhage and transfusion. These findings underline the need for careful obstetric management for this high-risk population.

The above-mentioned findings highlight the need for further, well-designed prospective studies and for optimizing diagnostic and therapeutic approaches in women with endometriosis who are planning pregnancy.

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POZVANA PREDAVANJA/
INVITED LECTURES

6.
REPRODUCTIVE CHALLENGES BEYOND THE CLINIC

Can Google Predict a Decline in Fertility? Digital Demography for Forecasting and Projections to 2050

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Objectives

This study investigates whether Google Trends, as a digital analytics platform, can function as a timely and reliable proxy for monitoring, assessing, and forecasting fertility trends. Focusing on Croatia as a case study, we explore the potential of "digital demography"—an emerging scientific subdiscipline that leverages online behavioural data—to detect demographic changes ahead of official statistics.

Our central hypothesis is that the number of monthly births in Croatia will decline by approximately 6% in 2025. This projection stems from patterns observed in real time search behaviour, which offers a decisive advantage over traditional demographic statistics. Official birth data from the Croatian Bureau of Statistics (DZS) are released with a minimum delay of eighteen months, limiting their usefulness for immediate policy interventions.

Digital demography harnesses "digital traces" left by the population through internet search activity, social media engagement, and other online interactions to anticipate demographic developments. The distinctive value of this method lies in its capacity to estimate key demographic indicators—including fertility levels and migration intentions—well before official datasets become available.

Our prior research has demonstrated a statistically significant correlation between fertility related keyword searches and subsequent birth rates. For example, during the COVID 19 pandemic in March 2021, Google Trends registered a notable decrease in searches for pregnancy tests. This suggested a reduced intention to conceive, which we hypothesised would lead to a significant decline in births in 2022. Eighteen months later, official DZS data confirmed this forecast. Encouraged by the accuracy of these findings, we extended our investigation into the post pandemic period to examine whether similar patterns persist.

Methodology

Using Google Trends, we modelled search frequencies for terms plausibly associated with pregnancy planning or confirmation, such as "pregnancy by weeks," "ovulation," "missed period," and "pregnancy symptoms." Additional attention was given to terms related to assisted reproduction ("IVF," "infertility," "infertility treatment"), given their potential influence on birth rates.

Results

Post pandemic data reveal sustained declines in all keyword groups indicative of pregnancy planning. Searches related to assisted reproduction also decreased, signalling a compounding effect on fertility trends. These concurrent declines suggest that the downward trajectory of fertility in Croatia may not merely be a transient, pandemic related anomaly but part of a longer term structural shift.

Implications

Based on our modelling, Croatia would require a total fertility rate of approximately 4.1 children per woman of reproductive age to maintain its current population size by mid century in the absence of immigration. The methodological framework presented here offers four principal contributions:

1) Early detection of demographic shifts, 2) Faster, data driven policy responses, particularly in population policy, healthcare resource allocation, and education planning, 3) Enhanced analytical precision through integration of real time digital data with demographic modelling, and 4) Potential for predictive analytics applicable across diverse demographic indicators.

Conclusion

By operationalising digital search behaviour as a leading indicator for fertility change, this research demonstrates that Google Trends can substantially augment demographic forecasting. The integration of digital demography into official statistical and policy frameworks could enable more agile, proactive measures to address the challenges of population decline, making it a valuable complement to traditional data sources in shaping long term demographic strategies.

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The Missing Lesson: FActs! on Fertility Awareness and Sexual Health Education in Croatia

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Fertility awareness is an essential part of reproductive health literacy. In Croatia, this is the missing lesson. Schools do not provide comprehensive sexuality education (CSE). What is taught is mostly confined to biology or religion classes, leaving many young people to enter adulthood without a full understanding of fertility and reproductive health.

To find out what teenagers in Europe actually know, Fertility Europe, together with experts from ESHRE, developed the digital game FActs!. This short, interactive game includes 15 questions on fertility, infertility, lifestyle factors, and sexually transmitted infections. Since its launch in 2023, it has been played more than 9,000 times in 50 countries, offering valuable insights into adolescents' knowledge of fertility and reproductive health.

The first four pilot countries were later joined by others, including Croatia. Croatian teenagers, however, scored lower than average. They were less familiar with facts about fertility and STIs, while questions on lifestyle factors, such as smoking and alcohol, were answered more successfully.

These results confirm what has long been observed: without proper education, teenagers turn to other, often unreliable, sources for information. Fertility awareness in adolescence is not about encouraging early parenthood or pressuring young people to have children. It is about ensuring they have access to clear, age-appropriate information so they can make informed decisions, understand what may affect their fertility, and seek help without stigma if and when they need it.

The Croatian FActs! results are a call to action. Fertility and reproductive health can no longer remain the missing lesson. Fertility education is not a luxury or a private matter, it belongs in public policy. Comprehensive sexuality education, including fertility awareness, should be integrated into school curricula. For this to happen, NGOs, educators, healthcare professionals, and policymakers must work together so that the next generation in Croatia grows up better informed, healthier, and better prepared for their future.

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Is there a need for a Common EU Regulation relating to Assisted Reproduction?

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Background

According to the European Society for Human Reproduction and Embryology (ESHRE), one in six couples worldwide experiences some form of infertility at least once during their reproductive lifetime. Legal regulation of assisted reproduction techniques (ART) has been developing since the late 1980s. Following the birth of the first child conceived outside the mother's body (UK, 1978), the first countries to adopt reproductive laws included Norway (1987), UK (1990), Austria (1992) and France (1994). A comparative analysis of early legislative documents in Europe (1980s–2000s) reveals major differences between national laws, primarily due to cultural, religious, and political reasons. Almost 45 years later, similar legal and ethical issues continue to arise in relation to ART. Questions regarding who should be entitled to access ART and under what conditions, remain subjects of debate. Additional concerns include the regulation of gamete and embryo donation and storage, surrogacy, donor anonymity, embryonic testing and embryonic research. Alongside, many scholars are still reluctant to fully address the legal status of the embryo and the scope of its rights, to avoid slippery slopes and ethical complexities related to the beginning and the end of life. Despite the advancements of modern ART, which has helped thousands of couples to conceive a child, such development also brings negative social impacts. These include commercialization (which limits access and may involve misleading marketing), reproductive tourism and the exploitation of women for oocyte donation and surrogacy. In such times, the present paper addresses the question of whether the time has come for a common EU legislation to be adopted regulating the field assisted reproduction.

Methods

Legislative analysis of original national legislation was used to reach the relevant legal findings, using grammatical, logical, structural and teleological interpretation, based on inductive-deductive model. Historical (longitudinal) and statical (de lege lata) method was used in comparative (cross-border) analysis.

Outcome

There is a need for a common EU regulation in the field of Assisted Reproduction. Harmonization in terms of minimal regulatory standards is crucial to grant equal and equitable access to all couples/individuals in Europe and to avoid different legal consequences deriving from cross border reproductive care. Special care should be dedicated to issues of gamete and embryo donation, mostly due to the ethical and safety reasons. The contribution proposes certain de lege ferenda solutions to be included in the future EU regulation.

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POZVANA PREDAVANJA/
INVITED LECTURES

7.
FROM STIMULATION TO IMPLANTATION: NAVIGATING THE COMPLEX PATH TO IVF SUCCESS

Hormonal analysis in IVF: benefits and dilemmas

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The activity of multiple hormones plays a crucial role in all stages of in vitro fertilization (IVF) and significantly affects treatment outcomes. Advances in gynecological endocrinology have led to more aggressive ovarian stimulation, the introduction of “freeze-all” strategies, and the increasing use of frozen embryo transfer (FET).

Hormone analysis during both the follicular and luteal phases of the IVF cycle provides benefits only when findings are correctly interpreted. Key factors include patient characteristics (age, BMI, ovarian reserve, basal hormones such as FSH, LH, E2, PRL, TSH, fT4) and prior treatments. Insulin resistance and hyperinsulinemia negatively impact IVF success and increase miscarriage risk. Elevated basal FSH and estradiol may indicate reduced ovarian reserve but are weak predictors in younger women with regular cycles.

Optimal ovarian stimulation aims to retrieve 9–15 mature oocytes with minimal risk of ovarian hyperstimulation syndrome (OHSS). Hormonal monitoring, alongside ultrasound, is essential for adjusting stimulation protocols. Markers of folliculogenesis include age, ovarian reserve, FSH, LH, estradiol, inhibins, and progesterone, while appropriate timing and type of trigger are critical for successful aspiration.

Challenges remain in analyzing the luteal phase and determining the best timing for FET. While evidence increasingly supports natural cycles, precise hormone monitoring is required. Modern IVF therefore relies on a combined approach of ultrasound and hormonal assessment to optimize outcomes.

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Discordance Between Serum Anti-Müllerian Hormone Concentrations and Antral Follicle Counts: Clinical Implications in IVF

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Anti-Müllerian Hormone (AMH) and antral follicle counts (AFC) share some features; they are both dependent on the action of granulosa cells, both reflect similar but not exact portion of total ovarian follicular pool. They both correlate well with the size of the remaining follicular cohort and predict response to ovarian stimulation (ROS) which makes them useful as markers for ovarian reserve. They both correlate negatively with age and positively with each other since small growing follicles included in the AFC are the main contributors to serum AMH concentration However, sometimes they do not align as expected and discordances, the deviation of the measured value of one parameter from the value expected from the measured value of another, between the two can be encountered. As both AMH values and AFC can be influenced by technical, physiological and exogenous factors, at first, the greatest emphasis was placed on technical issues, especially manual AMH assays and inter-operator variabilities of AFC. To address this question our group conducted a study with a single researcher on a large number of subjects to determine all AFCs and AMH was determined in a controlled setting of a single laboratory. The study aimed to investigate clinical and biochemical characteristics of the patients associated with AMH-AFC discordances. Our data expand the explanation of AMH–AFC disagreements from the technical issues only toward patient-specific differences in follicular production of AMH. In other words, when challenged against AFC, the serum AMH level is not only a quantitative but also a qualitative follicle marker, in relation with clinical and endocrine parameters.

Very low AMH and high AFC represent a situation where the use of high dose FSH according to AMH could end with the development of heavy OHSS. The AMH gene mutations can significantly impair serum AMH levels and might be discordant to AFC without affecting ROS. Caution should be taken when undetectable or severely reduced serum AMH levels are found in patients with high AFC. These findings also corroborate against for linking dose decisions to AMH level only.

AMH, AFC and age can be combined into the ovarian response prediction score (ORPI). ORPI had good prediction on oocyte yield, and the individualization of the ovarian stimulation regimen according to ORPI resulted in elimination of OHSS.

In conclusion, AMH-AFC discordance may lead to 1) misclassification of resting follicular pool leading to, misjudgement of ovarian reserve and delay in starting appropriate treatment and 2) misclassification of growing follicular pool leading to misjudgement of ovarian response, gonadotrophin dosage and suboptimal stimulation protocols.

Time to debate which biomarker is the best in predicting resting and growing follicular pool have passed. We should use all the information that we can obtain to design a therapeutic strategy

Not only in patients with AMH-AFC discordances, both AMH and AFC, together with other patient characteristics, will reduce the risk of misclassification and is likely to improve overall outcomes for our patients.

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The optimal number of oocytes we should aim with ovarian stimulation

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After decades of practice using IVF, it is now very clear that the 'one size fits all' approach may no longer exist. Individualization of treatment is not new to the field of medicine, although this concept is relatively fresh in reproductive medicine. There are new markers of ovarian reserve, the improvement in methodology for their measurement and the huge amount of clinical data have supported the view that individualization in IVF is the way forward. Ovarian response in IVF is a complex puzzle for which we now know the most important pieces. The optimal number of oocytes retrieved during controlled ovarian stimulation for in vitro fertilization (IVF) has been widely studied because it strongly influences success rates. To achieve success, we must use individualized protocols based on AMH, AFC, age and previous responses and try to avoid the OHSS sy. with appropriate diagnostics and trigger strategies.

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Follicle size at the time of trigger and oocyte maturation: does one size fit for all ovarian stimulation protocol

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It is well known that follicle size on the day of trigger correlates with the number of mature oocytes, number of embryos, pregnancy rates and live birth rates at the end. Although it has been widely accepted that follicles of 16–22 mm on the day of oocyte retrieval are most likely to carry mature oocytes, limited results exist to establish which exact follicle diameter on the day of trigger is most prominent to provide a mature oocyte. This is very important issue since too small follicles are most likely to yield immature oocytes while too large follicles could be empty or contain post-mature cells with supra-physiological progesterone levels that has negative impact on endometrium and implantation rate. The best time for triggering final oocyte maturation is not only dependent on follicle size but is also correlated to blood estradiol and progesterone level and patient previous protocols used for controlled ovarian hyperstimulation. Having all this on mind one could speculate that different ovarian stimulation protocols and even different trigger strategies could have different impact on follicle size, mature oocyte number and in vitro fertilization (IVF) success rate.

Controlled ovarian hyperstimulation is a crucial step of IVF procedures and is usually achieved through the use of long agonist protocol, antagonist protocol or minimal stimulation protocol mainly based on the physician's decision that includes potential benefits, risks and preferable outcomes of each treatment options. Although ESHRE guidelines do not specify the impact of different IVF protocols on preferable follicle size on the trigger day, few small studies indicated that the trigger injection could be applied later in agonist protocols when dominant follicles are of larger diameters as compared to antagonist cycles (2). The impact of the sizes of the rest of the follicle cohort could not be neglected either. Recently it has been shown that in minimal stimulation protocol with clomiphene citrate using the ovulatory trigger at follicle sizes above 20 mm resulted in larger number of total oocytes retrieved (3). Moreover, older patients (above 41 year) had benefits in terms of more mature oocytes and zygotes per retrieval when follicles were triggered at larger size. On the other hand patients of advanced age are also characterized by accelerated follicle growth, so it is reasonable to trigger the follicle of smaller size (around 15 mm) in natural cycles as compared to conventional cut-offs in order to prevent premature ovulation.

Weather the exact size of the follicle at the time of trigger have impact on oocyte maturation and IVF success rate in different IVF protocols is a question that should be elucidated in future studies.

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Novel Ovarian Stimulation Strategies in IVF: From Random Start and DuoStim to AI-Driven Individualised Protocols

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Recent advances in assisted reproduction have expanded the range of ovarian stimulation strategies available to clinicians, enabling more flexible, personalised, and time-efficient treatment plans. Among these, the Random Start protocol allows initiation of stimulation at any point in the menstrual cycle, proving particularly useful in urgent fertility preservation cases and in patients with limited time. The DuoStim approach, involving two stimulation cycles within the same menstrual cycle, has been applied to poor responders to maximise oocyte yield in a single month. Progestin-primed ovarian stimulation (PPOS) offers an alternative to GnRH analogues for the prevention of premature LH surge, while allowing for cycle scheduling flexibility without compromising clinical outcomes.

In parallel, the concept of individualised controlled ovarian stimulation (iCOS) has gained prominence, tailoring gonadotropin dosing and protocol choice according to ovarian reserve markers, previous cycle response, and patient-specific characteristics. Artificial intelligence (AI) is increasingly integrated into this process, using large datasets and predictive modelling to refine dose adjustments, improve protocol selection, and potentially enhance embryo quality and cumulative live birth rates.

This lecture will provide a concise, evidence-based overview of the clinical rationale, indications, and outcomes associated with Random Start, DuoStim, PPOS, and iCOS protocols, with an emphasis on how AI can augment decision-making in reproductive medicine. By combining innovative stimulation strategies with AI-assisted individualisation, IVF practice can move towards more efficient, patient-centred care, potentially improving both success rates and the overall treatment experience.

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Too Little or Too Much? The Progesterone Puzzle in Fresh and Frozen Embryo Transfers

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Aim of the work

To review the available evidence on serum progesterone concentrations measured either one day prior to or on the day of embryo transfer (ET), and to assess their impact on pregnancy outcome parameters.

Material and methods

A comprehensive literature review was conducted on studies published between 2015 and 2025, focusing on the association between serum progesterone levels and reproductive outcomes in IVF cycles, either in fresh or frozen embryo transfer treatments. Key databases included PubMed and selected studies included randomized controlled trials, prospective cohorts, and meta-analyses.

Results

Evidence consistently indicates that suboptimal progesterone levels (<10 ng/mL) in HRT-FET cycles are associated with reduced implantation, clinical pregnancy, and live birth rates—particularly when the vaginal route of administration is used, likely due to insufficient endometrial priming. In fresh embryo transfer cycles, elevated progesterone levels (>1.5 ng/mL) have been associated with premature endometrial advancement, resulting in embryo–endometrium asynchrony and poorer reproductive outcomes. Moreover, a decline in serum progesterone levels between the third and fifth day after embryo transfer may also negatively affect ongoing pregnancy outcomes, suggesting that progesterone stability post-transfer may be just as critical as its absolute value on the day of transfer.

Conclusion

In fresh cycles, minimizing elevated progesterone levels during the late follicular phase may help preserve embryo–endometrial synchrony and improve implantation outcomes. In HRT-FET cycles, adequate luteal support is critical for endometrial receptivity, particularly when vaginal progesterone administration is used. Furthermore, the majority of available evidence does not support a ceiling effect—suggesting that high serum progesterone concentrations do not negatively impact outcomes, especially in frozen cycles.

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Endometrial solutions for implantation failure: from theory to therapy

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Human embryonic implantation is a biological procedure based on two distinctive pillars, the embryo and the endometrium. Successful implantation is achieved due a continuous cross-talk between the embryo and the endometrium. The receptive endometrium is characterized by several changes that transform it to decidua. These changes are both morphological and functional and aim in establishing both a window of implantation and a window of selection. This secures the fact that only good quality embryos are to be implanted in a timely coordinated fashion. Several mechanisms have been proposed in order to depict implantation and implantation failure thereafter. Most of the mechanisms refer to the local microenvironment in terms of cellular populations' differentiation and local immunomodulation. In that aim several interventions have been reported in the literature aiming in modulating the local immune profile with promising results, including autologous platelet-rich plasma intrauterine administration, autologous properly-activated PBMC intrauterine administration, G-CSF administration, endometrial dysbiosis treatments and others. The current data is promising but rather weak in order for such interventions to be incorporated in contemporary practice. Further properly designed studies are therefore needed.

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Modulating the Reproductive Microbiome: The Role of Probiotics in Enhancing IVF Success

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The human gut microbiome comprises trillions of microorganisms that coexist with the host, significantly influencing metabolic, immune, and hormonal processes. Among these microbial communities, the estrobolome—a subset of gut microbiota genes responsible for estrogen metabolism, plays a pivotal role in women's health. Its activity affects systemic hormone levels, thereby influencing reproductive function, disease susceptibility, and overall homeostasis. This paper reviews current evidence linking the estrobolome and vaginal microbiota with reproductive outcomes and systemic health. Vaginal dysbiosis, often triggered by medications, medical interventions, lifestyle, or hormonal changes, can lead to common conditions such as vulvovaginal candidiasis (VVC) and bacterial vaginosis (BV). These disruptions are associated with a higher risk of urinary tract infections (UTIs), infertility, adverse pregnancy outcomes, and sexually transmitted infections (STIs). While scientific evidence increasingly supports the link between microbiota imbalances and health disorders, comprehensive data on the full impact of the vaginal and gut microbiome on women's health and fertility remains limited. Probiotics and lifestyle modifications, including the use of lactobacilli-based supplements, offer promising avenues for restoring microbial balance and improving clinical outcomes. The relevance of microbiome analysis in personalized medicine continues to grow, highlighting the need for a better understanding of microbial contributions to female health.

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POZVANA PREDAVANJA/
INVITED LECTURES

8.
PCOS: JOURNEY THROUGH THE MYSTERY OF AN ENIGMATIC SYNDROME

Challenges in the Diagnosis and Management of PCOS in Adolescents and Early Reproductive Age

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age and is frequently associated with obesity and insulin resistance. Due to its reproductive and metabolic complications, PCOS poses a significant health risk, affecting 3–11% of adolescent girls, depending on the diagnostic criteria used and the population studied.

Adolescence is marked by physiological pubertal changes—such as menstrual irregularities, hyperandrogenism, and polycystic ovarian morphology (PCOM)—that overlap with PCOS features in adults, making diagnosis challenging and increasing the risk of overdiagnosis when applying the Rotterdam criteria. International guidelines therefore recommend caution in diagnosing PCOS in young women less than eight years post-menarche. Menstrual irregularities should be defined in relation to the time since menarche. Mild to moderate acne and alopecia should not be included as diagnostic criteria. At this stage, ovarian maturity is incomplete, anti-Müllerian hormone levels are still rising, and the high prevalence of multifollicular ovaries limits the diagnostic value of PCOM. Nevertheless, early and accurate diagnosis is essential for identifying and preventing potential comorbidities such as type 2 diabetes, cardiometabolic abnormalities, non-alcoholic fatty liver disease, and psychological disorders, as well as for ensuring a smooth transition to adult care.

Lifestyle interventions—including exercise alone or in combination with dietary and behavioral strategies—are recommended for all women with PCOS to improve metabolic health, central adiposity, and lipid profile. Oral contraceptive pills remain the first-line pharmacological treatment, while metformin may be considered for adolescents at risk of, or with, PCOS for cycle regulation, acknowledging the limited evidence.

Adolescents presenting with only one diagnostic criterion should be classified as “at risk” and monitored regularly, with reinforcement of healthy lifestyle habits. Education on adolescent-specific PCOS diagnostic criteria is essential to improve clinical care.

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Metabolic Challenges of Polycystic Ovary Syndrome in Later Reproductive Age and Menopause

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Polycystic ovary syndrome (PCOS) is a lifelong endocrine and metabolic disorder, traditionally regarded as a reproductive condition but increasingly recognized as a systemic disease with implications well beyond fertility. From an evolutionary perspective, features such as insulin resistance, hyperandrogenism, energy storage propensity, and subfertility may once have provided adaptive advantages for survival in conditions of scarcity. In today's environment of caloric abundance and sedentary lifestyles, however, these traits have become maladaptive, predisposing affected women to obesity, metabolic dysfunction, and cardiovascular disease.

Across the lifespan, the metabolic consequences of PCOS evolve dynamically. During reproductive years, insulin resistance and compensatory hyperinsulinemia drive hyperandrogenism, weight gain, and an adverse cardiometabolic profile. More than 50% of women with PCOS are obese, typically with central adiposity, which exacerbates dysglycemia, hypertension, and atherogenic dyslipidemia. Meta-analyses demonstrate nearly threefold increased odds of type 2 diabetes and metabolic syndrome in women with PCOS compared with controls. By midlife, more than half of affected women may develop impaired glucose tolerance or overt diabetes, with significantly higher prevalence of hypertension and lipid abnormalities than the general population.

Emerging longitudinal data suggest that the relative risk trajectory changes after menopause. Hyperandrogenism often attenuates, while physiological menopause itself is associated with visceral fat accumulation and rising cardiometabolic risk in all women. As a result, postmenopausal women with prior PCOS appear to converge toward the risk profile of non-PCOS peers when analyses are adjusted for body mass index. Although obesity remains the dominant driver of cardiovascular events, studies show that women with PCOS continue to have lower HDL cholesterol, higher triglycerides, and increased markers of subclinical atherosclerosis into later life. However, large cohort studies report no significant excess of cardiovascular events in postmenopausal women with PCOS compared with BMI-matched controls, suggesting that weight and lifestyle are decisive modifiers of long-term outcomes.

The concept of developmental programming and possible transgenerational transmission of PCOS-related traits adds further complexity, underscoring the need for life-course prevention strategies. Clinical implications are clear: management of PCOS should not cease after reproductive years but should extend into midlife and beyond. Lifestyle intervention remains the cornerstone, with evidence that modest weight loss (5–10%) significantly improves insulin sensitivity and metabolic risk factors. Pharmacological measures such as metformin, GLP-1 receptor agonists, or statins should be considered according to individual risk profiles, while bariatric surgery may provide benefit in selected cases. International guidelines now emphasize systematic screening for diabetes, hypertension, and dyslipidemia across the lifespan of women with PCOS, and highlight the importance of early cardiovascular risk assessment and tailored interventions.

In summary, PCOS is a lifelong condition with evolving metabolic consequences. While excess cardiometabolic risk is most pronounced during reproductive and perimenopausal years, vigilance is required throughout life. Preventive strategies, sustained weight management, and proactive treatment of comorbidities can reduce long-term morbidity and improve quality of life for women with PCOS, even beyond menopause.

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From puberty to menopause: clinical interpretation of AMH in everyday gynecological practice

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Anti-Müllerian hormone (AMH) has emerged as one of the most informative biomarkers for female reproductive health, offering insights that span from the first signs of puberty to the onset of menopause. Produced by granulosa cells in small growing follicles, AMH plays a dual role: it restrains the recruitment of primordial follicles and modulates the ovary's sensitivity to follicle-stimulating hormone (FSH). Because of this, AMH is closely linked to the functional ovarian reserve, and its levels change in a highly predictable manner across the female lifespan.

In childhood, serum AMH is almost negligible, typically remaining below 7 pmol/L. A noticeable rise occurs two to three years before menarche, marking the initiation of follicular activity. During early adolescence, values generally range between 10 and 18 pmol/L. In certain conditions, such as Turner syndrome, an AMH concentration below 3 pmol/L from the age of six has been shown to reliably predict absent pubertal development, offering clinicians an early and precise diagnostic tool. By contrast, daughters of women with polycystic ovary syndrome (PCOS) often present with higher-than-expected AMH levels, reflecting their increased follicular pool long before clinical features of PCOS become apparent.

The reproductive years represent the period of highest AMH expression. Levels peak in the mid-twenties, with median values between 21 and 29 pmol/L, before beginning their steady decline. By age 35, typical concentrations fall to between 7 and 14 pmol/L, and beyond 40, most women record values below 7 pmol/L. Within the context of assisted reproduction, these numbers are highly informative: women with AMH above 25 pmol/L are at risk of hyper-response to stimulation, while those with levels below 5 pmol/L are more likely to demonstrate poor ovarian response. Despite these associations, AMH is not a direct predictor of natural fertility. Remarkably, spontaneous conception is still possible even when AMH approaches undetectable levels.

The timing of menarche also interacts with later reproductive potential. Data suggest that each year of delayed menarche increases the risk of secondary infertility by nearly 8%, and the risk of PCOS-related infertility by more than 16%. Conversely, early menarche is linked to accelerated ovarian aging, reflected by a faster decline in AMH and a lower ovarian reserve at younger ages.

As women approach menopause, AMH levels decline to below 1.5 pmol/L. A proposed cut-off of 0.09 pmol/L has been suggested as a biochemical marker of menopause. However, its clinical utility remains limited, with sensitivity and specificity values far too low for precise prediction.

Taken together, these findings confirm that AMH is a valuable marker throughout the reproductive life course. It supports decision-making in assisted reproduction, helps identify ovarian disorders, and provides women with meaningful information for fertility planning and preservation. Yet, it must also be emphasized that AMH is not a definitive predictor of either spontaneous fertility or the exact timing of menopause. For consistent and reliable use, standardized assays and age-specific reference ranges are essential. In daily gynecological practice, AMH should therefore be viewed not as a solitary determinant but as part of a broader framework for assessing female reproductive health.

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Multidisciplinary holistic approach to treating PCOS at a private gynecology clinic in Slovenia

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Objectives

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in reproductive-age women. Its key features are hyperandrogenism, polycystic ovarian morphology (PCOM), menstrual irregularities, central obesity, and psychological disturbances, often linked to weight gain, obesity, and infertility concerns. Guidelines recommend lifestyle modification as first-line therapy. While various pharmacological options exist, non-hormonal management is most frequently chosen. Given its complexity, PCOS requires a multidisciplinary, long-term approach. This study analyzed clinical characteristics and treatment preferences of women with PCOS to identify effective strategies in a private gynecological diagnostic institution in Slovenia.

Methods

This observational study included 246 women diagnosed with PCOS according to the International Evidence-Based Guideline for PCOS between January 2021 and September 2024. Clinical history, examination, ultrasound and laboratory testing (biochemical, metabolic, and hormonal profiles) were performed. Data on menstrual irregularities, hyperandrogenism, hirsutism, PCOM, psychological disorders, BMI, age and therapy were collected and statistically analyzed.

Results

Mean patient age was 27.8 years; 56.9% had BMI ≥ 25, 43.1% were lean. In phenotype A, 11% had BMI ≤ 25, while 86.7% of phenotype D had BMI ≤ 25. The mean age in the subgroup with BMI ≥ 30 was 28.3 years. Hirsutism occurred in 72.2% overall and in all women with BMI ≥ 30. Menstrual irregularities affected 86.1%, most often oligomenorrhea (49%). All women with phenotype A, B, and D reported irregular cycles. PCOM was found in 92.1%, confirmed by ultrasound. Psychological problems were present at 34.7%, highest in phenotype A (45.9%), lowest in phenotype D (21.7%). Binge eating disorder was reported by 35.5% of phenotype A. The HOMA index was abnormal in 84% of phenotype A, 29.4% of phenotype D, and 91.3% with reduced SHBG.

Regarding therapy, 59.7% of phenotype A women chose non-hormonal treatment, 8.3% hormonal therapy, and 30.3% supplements only. Metformin 2000 mg daily was selected by 84.6% of this group. In phenotype D, 25% opted for non-hormonal therapy, 23.3% hormonal therapy, and 51.7% supplements. Low dose combined hormonal contraception was the most frequently prescribed hormonal option; two women used spironolactone 100 mg daily.

Conclusions

Women with PCOS generally accept multidisciplinary, evidence-based programs with long-term follow-up, yet non-hormonal strategies remain predominant. Hormonal therapy is often rejected, even in our setting. Diet and supplements are the most welcomed interventions. Classical phenotype patients typically reach obesity by age 28, but one third decline effective options for reduction of weight and insulin resistance. Conversely, less than one quarter of phenotype D patients accepted hormonal therapy for menstrual irregularities. These findings highlight the need for better education and awareness of PCOS in the general population and among affected women.

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PCOS and folate: a small molecule, a big impact

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Folate deficiency in women with polycystic ovary syndrome (PCOS) plays an important role in the metabolic and reproductive imbalances associated with the syndrome. Folate, a vital B9 vitamin, is involved in regulating homocysteine levels and DNA synthesis, both critical for normal ovarian function and reproductive health. There are numbers of studies conducted and have confirmed the fact that the homocysteine plasma level is significantly raised in women with PCOS. Studies show that low folate levels can lead to increased homocysteine, which is often linked to insulin resistance, female infertility, and a higher risk of cardiovascular complications in PCOS. Folate independently contributes to controlling metabolic parameters and reproductive health, particularly through homocysteine regulation and supporting normal ovarian cell functions.

Supplementation with folic acid in PCOS patients helps reduce metabolic risks and improve reproductive outcomes. The main benefits of folic acid supplementation include lowering elevated homocysteine levels, which are commonly observed in PCOS and associated with increased cardiovascular risk. It also helps reduce inflammatory markers and oxidative stress, important factors in metabolic health. Additionally, folic acid aids in DNA synthesis and the development of healthy oocytes, which supports ovulation regulation and increases the chances of pregnancy. Some research also indicates improvements in glucose and lipid metabolism, decreased insulin resistance, and reduced inflammation following folic acid supplementation.

However, more research is needed to fully understand the role of homocysteine in PCOS and to clarify its relationship with insulin resistance, androgen levels, and other biochemical parameters. A clearer understanding would help develop better treatment options, reducing women's short- and long-term risks of cardiovascular disease, type 2 diabetes, infertility, recurrent miscarriages, and psychological disorders. Achieving these goals would significantly enhance the quality of life for women living with PCOS.

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Inositols – assistance with PCOS and support for fertility

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In sexually active couples who are planning pregnancy it is recommended that a woman undergo an infertility evaluation if she has not been able to conceive a successful pregnancy within 12 months of unprotected intercourse if younger than 35 or after 6 months of unprotected intercourse if she is older than 35 years of age. Infertility is problem for 15-20% of couples worldwide. Female infertility can be caused by ovulation disorders in around 25% women. Polycystic ovary syndrome (PCOS) is not just a fertility problem but depending on reproductive age, it creates various issues such as irregular menstrual cycles, acne, hirsutism, seborrhea, spontaneous miscarriages, diabetes, and hypertension during pregnancy, metabolic syndrome and increased incidence of malignant diseases in menopause. The pathophysiology of PCOS is still not fully understood. Among the other causes genetic testing is being intensively researched lately. The treatment of PCOS is also not fully satisfactory. Oral hormonal contraception is not always an option, so inositols are becoming very promising. Inositol is a form of alcoholic sugar. It can be found in certain foods, including meat, fruits, corn, beans, grains and legumes but typical diet contains usually only 1-2gram of inositol and body is not able to produce sufficient amount so recent research suggests that taking an inositol supplement may have many health benefits, and especially for PCOS and women infertility. In Catafertyl for women there are the best ratio of the two most important inositols myo-inositol and d-chiro-inositol 40:1. It also contains other supplements like active folic acid quatrefolic, vitamin D, zinc and other, in unique combination of ingredients for women hormonal balance and fertility. There is also Catafertyl for men. Men infertility is arising problem and app. 30% is still of unknown origin. More than 50% of infertile men have elevated markers of oxidative stress, and the addition of antioxidants in the diet significantly improves sperm results. In Catafertyl there is 600mg N-acetyl-L-cysteine (NAC). NAC is a derivative of L-cysteine, primarily used as an antioxidant. It contributes to the synthesis of glutathione and can help replenish the depleted amounts of glutathione caused by oxidative stress and inflammation. Additionally, NAC is also capable of directly reducing oxidative stress. Catefertyl also contains L-arginine which is one of the most metabolically versatile amino acids important for the production of nitric oxide (NO), which is a vasodilator, thus improving circulation and supplying tissues with blood and oxygen. It also actively participates in the formation of sperm. A deficiency of L-arginine causes a disruption in sperm metabolism, leading to reduced motility and loss of spermatogenesis. Studies have shown that L-arginine supplementation increases semen volume, concentration, motility, and morphology. L-citrulline is a natural precursor to L-arginine. Catafertyl for him also contains other supplements like vitamin C, zinc and other, in unique combination of ingredients for men hormonal balance and fertility.

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POZVANA PREDAVANJA/
INVITED LECTURES

9.
CONTRACEPTION – BETWEEN MYTHS AND FACTS

Do we need an individualized approach or standardized tests for all users of oral hormonal contraception?

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There are several guidelines and recommendations worldwide on the use of all forms of hormonal contraception, both among healthy populations without risk factors and among those considered at risk.

- The World Health Organization (WHO) has provided instructions and a classification of medical conditions for the use of oral contraceptives.
- According to these guidelines, diseases and conditions are rated categorically from 1 to 4 depending on the risk of occurrence.

CATEGORY 1 - no restrictions on the use of OHK.

CATEGORY 2 - conditions in which the benefits of using oral contraceptives outweigh the theoretical or proven risks.

CATEGORY 3 – relative contraindications.

CATEGORY 4 – absolute contraindications

Standardized tests in users of oral hormonal contraceptives:

history and clinical examination, detailed history (family history of thrombosis, cardiovascular disease, migraines, smoking, etc.), assessment of risk factors (smoking, hypertension, obesity), measurement of blood pressure (before and during therapy). Laboratory tests (not always required but often recommended): complete blood count (CBC): To monitor the general condition, possible anemia or disorders. Lipid profile: oral contraceptives can affect lipid levels (cholesterol, LDL, HDL, triglycerides). liver function (AST, ALT, GGT): Contraceptives can affect the liver. Blood glucose or HbA1c: Especially in women with a predisposition to diabetes. Thrombophilia status: not routine, but if there is a family history of thrombosis, it may be done (eg tests for thrombophilia factors). TSH (thyroid): If there are symptoms or suspicion of thyroid disorders.

Standardized tests: they are useful for basic screening of all OHK users, especially in primary health care. They enable early detection of potential risks (eg hypertension, lipid disorders, liver disorders).

Individualized approach: necessary for women who have specific risk factors or health problems.

Conclusion

The best practice is a combination of both approaches. Standardized tests for all users as an initial basic screening. Individualized approach and additional tests in patients with identified risks or symptoms, This achieves a balance between efficiency and safety.

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Do we need an individualized approach or standardized tests for all users of hormonal contraception? Cons: Testing do not increase safety, create a false impression of increased risk.

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Aim

A review of published evidence (supporting - not supporting) focused to general screening before using hormonal or other contraception.

Certain contraceptive methods may increase the risk of adverse events for women with certain medical conditions, including some women with diabetes, hyperlipidemia, liver disease, cervical cancer, sexually transmitted infections (STIs), human immunodeficiency virus (HIV) and hereditary thrombophilia's. There is a dilemma as to whether universal screening affects health risks in users of hormonal contraception.

Result

The systematic review did not identify any relevant direct evidence about general testing or not.

Conclusion

There is little value in screening for these conditions in asymptomatic women prior to initiation of contraceptive methods due to the low prevalence of these conditions among women of reproductive age. Current evidence supports individualized approach.

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Venous Thrombosis and Hormones: Where Science Ends and Fear Begins

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Combined oral contraceptives (COCs) have periodically been the subject of public health scares over venous thromboembolism (VTE) risk. Since the 1960s, four major "pill scare" waves – in 1961–63, 1970, 1995, and 2010–13 – have shaped the evolution of contraceptive safety measures. Early high-estrogen pills (e.g. Enovid®) were linked to VTE in case reports, prompting substantial reductions in estrogen dose. A second wave (Nelson hearings, 1970) further lowered doses and strengthened pharmacovigilance. In 1995, media coverage of a study reporting a two-fold higher VTE risk with "third-generation" progestins led to public panic, a ~20% drop in pill use, and increased unplanned pregnancies. This crisis underscored the need for clearer communication of relative vs. absolute risk. The most recent scare (2010–2013), focused on drospirenone and other new progestins, triggered regulatory reviews by FDA and EMA, but ultimately confirmed that the benefits of all COC formulations outweigh their risks. These episodes drove improvements in pharmacovigilance, including mandatory post-authorization safety studies (PASS) and enhanced surveillance via EudraVigilance in the EU.

It is now recognized that thrombotic risk with COCs is multifactorial. Baseline VTE incidence in young women is low (≈2–5 per 10,000 women-years), but genetic thrombophilias (e.g. factor V Leiden), age, obesity, smoking, and other comorbidities can synergistically elevate risk. Use of any COC roughly triples VTE risk versus non-use, though the absolute risk remains small. Notably, pills containing desogestrel, gestodene, or drospirenone (3rd/4th-generation progestins) have about 1.5–2 times higher VTE risk than those with levonorgestrel, especially at higher ethinylestradiol (EE) doses. Importantly, recent data from a large PASS study demonstrated that the combination of EE with chlormadinone acetate (CMA) carries a VTE risk comparable to the standard EE + levonorgestrel formulation, underscoring the heterogeneity among progestins. Mechanistically, estrogenic COCs induce a prothrombotic state marked by acquired resistance to activated protein C (APC). This effect correlates with the estrogen dose and type: EE exerts a strong first-pass hepatic effect, upregulating clotting factors, whereas natural estrogens like estradiol valerate (E2V) produce a milder hepatic impact. Estetrol (E4), a novel native estrogen with selective tissue activity, has minimal influence on liver protein synthesis and hemostatic balance. Accordingly, E4-containing COCs show negligible changes in APC resistance and only a minimal estimated increase in VTE risk. Early clinical data on an E4/drospirenone pill are promising: two large trials reported a VTE incidence of ~3.6 per 10,000 women-years (comparable to older low-dose EE/LNG pills), with no serious sequelae. Ongoing PASS studies will further clarify its safety profile.

Current clinical recommendations emphasize individualized risk assessment and risk minimization. International guidelines (WHO, CDC, EMA, ACOG, FSRH) advise against routine thrombophilia or APC-resistance screening before prescribing COCs, focusing instead on thorough personal and family history evaluation. In women with multiple risk factors or hereditary predispositions, progestin-only or non-estrogenic methods are preferred. For most users, choosing a preparation with the lowest effective estrogen dose and a lower-risk progestin is prudent. Newer options containing E2 and especially E4 may offer similar efficacy with potentially lower thrombotic impact, expanding choices for risk-conscious prescribing.

In conclusion, six decades of experience have improved our understanding of COC-related VTE. Effective public health messaging is paramount to place the VTE risk in context – for instance,

highlighting that pregnancy itself carries a higher thrombotic risk and that the absolute VTE risk with modern pills is very low. Clear, evidence-based communication can prevent undue alarm from sensationalized reports and avoid the adverse outcomes of pill scares (such as unintended pregnancies and abortion surges). Ongoing pharmacovigilance and education ensure that women can confidently reap the benefits of contraception while risks are minimized and transparently communicated.

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Let's Be Objective – Evidence from Clinical Studies

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The role of hormonal contraception (HC) in modern gynecological endocrinology is indisputable. However, a growing body of clinical and scientific studies investigates its potential impact on women's mental health and sexual function. This lecture aims to objectively present evidence from clinical trials and systematic reviews, considering the diversity of hormonal formulations, biological individuality, and psychosocial context of users.

Numerous studies have demonstrated an association between specific combined contraceptives and an increased incidence of depressive and anxiety symptoms, as well as decreased sexual desire, particularly among adolescents and women predisposed to affective disorders. On the other hand, in women suffering from pronounced premenstrual symptoms or dysmenorrhea, HC may exert a mood-stabilizing effect and indirectly improve sexual functioning.

Modern hormonal contraceptives differ substantially in estrogen and progestin composition. Preparations containing ethinylestradiol and antiandrogenic progestins (such as drospirenone) have been linked to higher risks of mood disturbances and reduced libido. Conversely, newer contraceptives containing physiological estrogens (estradiol valerate, estetrol) combined with more "neutral" progestins (nomegestrol acetate, levonorgestrel) demonstrate improved tolerability profiles regarding both emotional stability and sexual function.

In clinical practice, most combined contraceptives still rely on synthetic estrogen (ethinylestradiol), but newer generations are increasingly shifting toward more physiological estrogens and neutral progestins to minimize unwanted effects on mental health and sexual well-being.

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Hormonal Contraception and its impact on mental health and sexual function - Balancing benefits, risks, and the need for personalization

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Hormonal contraception represents a major component of family planning and reproductive medicine. Despite the wide availability of hormonal preparations such as oral contraceptive pills, vaginal rings, patches, and intrauterine devices (IUDs), a significant proportion of women avoid their use due to concerns about side effects, including mood changes, weight gain, altered libido, and impact on fertility. Globally, the combined oral contraceptive pill (COC) remains the most used method. Modern COCs containing ≤35 µg ethinyl estradiol (EE) appear to have fewer mood-related symptoms compared with older high-dose formulations (≥50 µg). While EE is the most prevalent estrogen, alternatives such as estradiol valerate and estetrol are available. In contrast, the spectrum of available synthetic progestins is broader, differing in potency, androgenicity, and tolerability, though contraceptive efficacy remains comparable. Third- and fourth-generation progestins are generally less androgenic, but the clinical significance for mood and sexual function is still debated.

Estrogen and progesterone modulate neurochemistry and neurotransmitters such as serotonin, dopamine, and GABA. Estrogen demonstrates neuroprotective effects and may reduce the risk for cognitive decline and affective disorders. Moreover, COCs can improve premenstrual dysphoric disorder (PMDD) in some women. Conversely, evidence also indicates that both estrogen and progesterone may cause mood changes and enhance depressive symptoms. Mood disturbance is one of the most frequently mentioned reasons for therapy discontinuation. Mechanisms likely include direct pharmacologic effects on neurotransmitter systems as well as indirect psychological influences of contraceptive use.

Concerns have been raised that the ovarian androgen suppression associated with COC use could negatively affect libido. Although most studies report minimal overall impact on sexual function, results are mixed, and some women still complain of decreased sexual function. A systematic review found that 22% of women experienced increased desire and 15% decreased desire after starting COCs, while most reported no change. Vaginal dryness, reported by some oral contraceptive users, may further negatively affect desire and comfort.

Personalized selection of contraceptives is essential, since individual formulations show different psychosexual effects. Nomegestrol acetate with 17-β-estradiol in women with mood disorders appears to show more tolerability. By contrast studies have shown progestogen-only preparations may worsen depressive symptoms in predisposed women. Although levonorgestrel-releasing IUDs are often considered to have localized effects, cohort data suggest possible associations with new-onset depression, anxiety, and sleep disturbances.

Overall, evidence highlights the need for individualized counseling, shared decision-making, and close monitoring to balance the benefits of effective contraception against potential adverse effects on mental health and sexual function.

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POZVANA PREDAVANJA/
INVITED LECTURES

10.
ONE GOAL, ONE TEAM: CLINICIANS AND EMBRYOLOGISTS TOGETHER

The Path to Precision: Clinical Evidence Shaping Ovarian Stimulation Today

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Post-hoc and individual patient data (IPD) meta-analyses are valuable tools for shaping contemporary ovarian stimulation strategies. This presentation leverages data from phase II and III randomized controlled trials and pooled IPD analyses involving over 1,700 patients from multiple international studies. Targeted post-hoc subgroup analyses explored outcomes in patients with high, normal, and low ovarian reserve. Meta-analytic techniques were used to examine the relationship between oocyte yield and live birth rates in both fresh and cumulative cycles. Additionally, these analyses provided insight into other timely clinical questions—namely, whether a freeze-all approach improves pregnancy outcomes and whether endometrial thickness remains a meaningful predictor of cycle success. Post-hoc and IPD meta-analyses offer nuanced perspectives that enhance our understanding of ovarian stimulation strategies. These data underscore how robust secondary analyses can refine clinical decision-making and support the evolution of precision medicine in reproductive endocrinology.

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Clinical evidence shaping ovarian stimulation. What would you do?

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Individualized ovarian stimulation has become a core concept in assisted reproduction, moving away from a “one size fits all” model toward tailored strategies that incorporate patient specific factors such as anti Müllerian hormone (AMH) levels, antral follicle count (AFC), age, body mass index (BMI), and previous ovarian response. This individualized approach aims to optimize gonadotropin selection and dosing in order to maximize cumulative live birth rates while minimizing treatment risks and burden.

Highly purified human menopausal gonadotropin (HP hMG), which provides follicle stimulating hormone (FSH) together with LH bioactivity derived from human chorionic gonadotropin (hCG), has been shown to enhance follicular steroidogenesis and support late follicular maturation. A recent systematic review and meta analysis highlighted that adding LH activity—whether through recombinant LH or hCG derived LH in HP hMG—confers clinical benefit in specific subgroups, including poor ovarian responders defined by Bologna criteria with high PROsPeR scores, hypo responders to recombinant FSH (POSEIDON groups 1 and 2), and women of advanced reproductive age up to 40 years (POSEIDON groups 2 and 4).

In parallel, individualized recombinant FSH dosing with follitropin delta has been evaluated across several levels of evidence. A recent systematic review and meta analysis demonstrated that, in women with normal ovarian reserve as well as in poor responders, stimulation with follitropin delta resulted in clinical pregnancy and live birth rates comparable to or higher than those achieved with follitropin alfa and beta, while the incidence of ovarian hyperstimulation syndrome (OHSS) was significantly lower in women with normal and high ovarian reserve. Some studies focused specifically on POSEIDON classified poor responders, showing improved oocyte maturation and a more efficient oocyte to dose ratio with follitropin delta compared with prior cycles using follitropin alfa or beta.

A large real world evidence (RWE) analysis further confirmed these findings, showing that the AMH and body weight–based dosing algorithm of follitropin delta achieved higher cumulative live birth rates with a favorable safety profile compared with follitropin alfa and beta. A dedicated cost effectiveness study also demonstrated that follitropin delta, when compared with follitropin alfa and beta, provided similar or improved clinical outcomes with significantly reduced total gonadotropin requirements and overall treatment costs. These data underscore that, beyond efficacy, economic considerations and resource optimization are increasingly relevant in modern reproductive medicine.

Taken together, these findings show that optimizing both the type and the dose of gonadotropin is essential: HP hMG with LH activity is advantageous in well defined subpopulations, while AMH and weight based follitropin delta protocols offer improved safety, efficiency, and, in several analyses, superior effectiveness compared with conventional follitropin alfa and beta. Moving beyond a one size fits all model to an individualized, evidence based approach is now central to achieving better clinical outcomes, minimizing risks such as OHSS, and ensuring more cost effective use of gonadotropins in assisted reproduction.

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SET vs DET from the embryologist's perspective

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In the early years of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), clinicians may have transferred all available embryos into the uterus because of low implantation rates (IR), suboptimal embryo culture and cryopreservation procedures. However, with medical and technical improvements in Assisted Reproductive Technology (ART), the transfer of multiple embryos led to a greater number of high-order multiple pregnancies compared to spontaneous pregnancies. Multiple pregnancies are associated with a wide range of maternal and neonatal complications (low birth weight, preterm birth, higher risks of neonatal mortality...). Compared to double embryo transfer (DET), single embryo transfer (SET) has a lower live birth rate due to several factors, but if the cumulative live birth rate is taken into account, the rate is similar for subsequent frozen SET cycles. The core advantage of SET is that it minimizes the risks of multiple births. Still, the decision (DET vs SET) should be made by considering individual patient factors, embryo quality (controversial), rank of treatment and the goal of achieving a healthy (singleton) pregnancy.

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Freeze for all, anytime

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Aim of the study

"Freezing for all, anytime" represents a paradigmatic revolution in reproductive medicine. It establishes a new imperative, every person has the right to preserve their fertility, regardless of age, socioeconomic status, medical indication or geographic location. This concept goes beyond the traditional medical model and places cryopreservation as a fundamental reproductive right that must be available to everyone. Cryopreservation technologies are no longer experimental, they are now established, safe and effective methods (more than 90% survival of gametes and embryos) that allow individuals to take control of their reproductive future. The percentage of use of the social egg freezing and "Freeze all strategy" is growing globally every year and reflects a growing social consensus on the need for reproductive autonomy.

The primary objective of this paper is to argue for the universal application of the concept of "Freezing for all, anytime" as a fundamental reproductive right that must be available to all members of society.

Materials and Methods

This paper uses multidisciplinary data that combines clinical evidence, economic analysis, and ethical arguments to fully advocate for the aforementioned approach. Numerous studies demonstrate the consistent superiority of cryopreservation technologies.

Results

Numerous studies irrefutably demonstrate that vitrification is a superior technology that must be available to all. The oocyte survival rate (90-97%), the live birth rate (32-40% per transfer), and the safety profile justify universal access, and show that freezing all is as successful as fresh cycles. "Freeze for all, anytime" is a safe method without increased risk to the mother or the newborn. Numerous benefits have been documented in the literature that advocate universal use. Thus, we can find that all patients benefit from a reduction in OHSS (78%). The population requiring PGT-A shows a 19% benefit (success rate 80% vs 61%). This makes genetic screening more accessible when combined with "Freeze all". Endometrial receptivity in frozen cycles enables better outcomes for all categories of patients, as well as for those with high progesterone values at the end of the follicular phase, and some uterine pathologies such as polyps, adenomyosis, etc. In addition to the above, social freezing becomes a fundamental right, not a privilege, as evidenced by the exponential growth of 39.2% per year on a global level. Society clearly expresses its desire for reproductive autonomy. Universal approach results in positive social benefits. Key advantages: increased women labour participation through reproductive security, optimized family formation timing, reduced healthcare burden etc.

Conclusion

"Freezing for all, anytime" is not a utopian vision, but an achievable reality that requires political will and social commitment. Vitrification is established as a superior technology. The ethical imperative is clear, reproductive rights are human rights. This concept represents the evolution of human rights, from basic reproductive freedom to comprehensive reproductive security. The final proof of all of the above is simple. If the technology exists, if it is safe, effective and if society benefits, access must be available and unique for all. And finally, "Freeze for all, anytime" is not just a populist policy, it is a moral imperative for any society that truly stands for equality, justice and human dignity, because reproductive autonomy is a human right, not a privilege.

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Do all patients need fertility preservation?

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Breast cancer remains the most common malignancy in women worldwide, with more than 2.3 million new cases diagnosed in 2022 and projections indicating further growth, particularly among women of childbearing age. Advances in screening and treatment have reduced mortality, yet the increasing population of survivors shift attention towards survivorship and quality of life. Fertility is a key concern, as cytotoxic therapies, radiotherapy and targeted agents may damage ovarian reserve, while prolonged endocrine treatment in hormone receptor-positive tumours further delays childbearing. In addition, younger patients often present with biologically aggressive tumours and lower survival rates, complicating reproductive decision-making. The question is not whether all patients need fertility preservation, but how to provide tailored strategies. According to ESHRE and ASRM recommendations, decisions should consider age, ovarian reserve, tumour subtype, treatment type and dose, comorbidities, relationship status, and reproductive goals. Oocyte and embryo cryopreservation are the standard of care when time allows, whereas ovarian tissue cryopreservation or ovarian suppression may be considered in selected or urgent cases. Individual risk assessment requires a multidisciplinary approach, integrating oncologist, reproductive specialists, and psychologists to balance oncological safety with fertility potential. Psychological support plays a crucial role, as fertility concerns are among the strongest predictors of distress in young survivors. Timely oncofertility counselling, clear communication of options and risks, and structured referral pathways are essential to empower women with informed choices. Beyond clinical aspects, equitable access to fertility preservation services and supportive infrastructure are vital to ensure that all women can benefit, regardless of socioeconomic status or geography. It is therefore time to rethink fertility preservation in breast cancer patients. Not every woman will ultimately peruse fertility preservation, yet every woman deserves urgent oncofertility counselling and an individualised, multidisciplinary strategy to protect both survival and reproductive autonomy, ensuring the survivorship also means quality of life.

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Do we need anti-estrogen therapy in the stimulation protocols?

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Fertility preservation (FP) in women with breast cancer presents a complex clinical challenge, particularly because many tumors are hormone receptor (HR) positive. Controlled ovarian stimulation (COS), a key step in FP, leads to transient supraphysiological serum estradiol levels. Although these elevations are temporary, they could theoretically stimulate the proliferation of malignant cells. To date, however, there are no data demonstrating an adverse oncological effect of COS for FP in women with breast cancer.

To minimize potential harm from elevated estradiol levels, innovative stimulation protocols have been developed. These include the co-administration of aromatase inhibitors or selective estrogen receptor modulators during ovarian stimulation. Such approaches are frequently used in clinical practice to reduce estrogen exposure while maintaining oocyte yield. Current evidence indicates that the number of cumulus–oocyte complexes (COCs) retrieved, as well as the number of oocytes or embryos cryopreserved, is not compromised when alternative protocols incorporating anti-estrogen therapy are used in women with breast cancer.

Most available evidence comes from observational studies, such as retrospective or prospective cohort studies, that follow patients who have chosen FP interventions and compare their oncological outcomes with those of a matched control group who have not undergone FP. The recent Female Fertility Preservation guideline from ESHRE emphasizes that further research is needed on the long-term effects of ovarian stimulation with tamoxifen or letrozole co-administration.

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POZVANA PREDAVANJA/
INVITED LECTURES

11.
8th CONGRESS OF CROATIAN SOCIETY OF CLINICAL EMBRYOLOGISTS WITH INTERNATIONAL PARTICIPATION

Advanced maternal age affects FET susceptibility to high oxygen environment

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The oocytes of older women often have chromosomal abnormalities. However, it is difficult to prove other age-related cellular deficiencies, such as stress tolerance, due to suboptimal laboratory conditions. It has been shown that preimplantation embryos can experience stress when atmospheric oxygen concentrations are used in embryonic culture conditions.

In this animal model study, embryos obtained by in vitro fertilization (IVF) from oocytes of two- and eight-month-old mice were used. The embryos were vitrified and thawed at the 6–8-cell stage and cultured in a low oxygen concentration (5%) (LOT) and high oxygen concentration (20%) (HOT) environments. We evaluated the morphology, apoptosis, in vitro proliferation capacity of the inner cell mass (ICM), and pluripotency of the embryos.

Embryos from older animals cultured in HOT had reduced fertilization capacity, lower survival rates after thawing, increased apoptosis, and reduced complete ICM growth compared to similar embryos cultured in LOT. The study demonstrates that embryos derived from oocytes of older females are more susceptible to oxidative stress than embryos from younger females and have lower stress tolerance under suboptimal atmospheric conditions.

The findings of this animal study should also be verified in human embryos, although recommendations advise against culturing human embryos at atmospheric oxygen concentrations.

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Mitochondrial DNA mutation in human oocytes

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Aim of the study

Mitochondrial DNA (mtDNA) mutations in human oocytes represent a critical factor in reproductive biology, with profound implications for maternal inheritance, embryonic development, and the onset of mitochondrial diseases. Unlike nuclear DNA, mtDNA is exclusively maternally transmitted and lacks robust repair mechanisms, making it particularly vulnerable to damage and mutation accumulation over time.

Material and Methods

This presentation aims to give an overview on the potential association of mitDNA with IVF outcome with particular focus on mutations. It should explore the origin and dynamics of mtDNA mutations during oogenesis, emphasizing the role of oxidative stress and maternal aging.

Results

Mitochondrial DNA content reflects oocyte viability and fertilisation outcome. Higher rates are obviously better because it reflects higher available ATP levels. Mutations, however, can compromise mitochondrial function, leading to impaired ATP production, increased reactive oxygen species (ROS), and altered cellular metabolism—factors that may contribute to reduced oocyte quality and fertility. Not to forget to mention that mitochondrial deseases can be trasmitted. The implications of these findings for preimplantation genetic diagnosis (PGT), mitochondrial disease prevention, and fertility preservation strategies are discussed.

Conclusion

By integrating molecular insights with clinical perspectives, this presentation aims to deepen our understanding of mitochondrial genetics in human reproduction and highlight future directions for research and therapeutic innovation.

Decoding embryo development: patient and embryo factors shaping morphokinetics

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Aim of the study

To explore how patient-related (male BMI, semen quality and sperm origin) and embryo-related (chromosomal constitution) factors shape embryo morphokinetics and reproductive outcomes using large-scale time-lapse data.

Materials and methods

We retrospectively analyzed 30,384 embryos from ICSI cycles performed between 2018-2024 across multiple centers. Three independent analyses were conducted to explore determinants of embryo morphokinetics and reproductive outcomes. The first evaluated the impact of male BMI in 7,846 embryos from oocyte donation cycles. The second addressed the influence of semen quality and sperm origin in 10,623 embryos from cycles using either ejaculated or testicular (TESE) sperm. The third examined the relationship between embryo ploidy and developmental dynamics in 11,915 embryos from PGT-A cycles. Morphokinetic parameters were annotated with time-lapse systems and analyzed using regression models, Cox survival curves, clustering, and multivariate analyses adjusted for relevant confounders.

Results

Embryos from obese men showed delayed early cleavage divisions (t2-t5), poorer inner cell mass quality, and higher miscarriage rates relative to those from normal-weight males (13.5% vs 9.5%, respectively), with a trend toward reduced live birth rates (36.5% obese vs 46.7% normal-weight). Semen parameters also shaped developmental potential. Poor quality samples were linked to lower fertilization and blastocyst rates, particularly in TESE cycles. Although TESE-derived embryos cleaved faster (from tPNf-t5) than those from ejaculated sperm, the resulting blastocysts were of poorer quality and led to significantly lower cumulative implantation, pregnancy, and live birth rates. Chromosomal constitution further influenced developmental dynamics. We identified five distinct developmental trajectory groups, each independently associated with embryo ploidy. Specifically, embryos with chromosomal losses or multiple abnormalities showed marked delays in reaching the blastocyst stage, while those with chromosomal gains followed timelines similar to euploid embryos.

Conclusion

Our findings demonstrate that both patient- and embryo-related factors shape developmental dynamics, each with varying degrees of magnitude. While paternal BMI and semen quality exert measurable effects, sperm origin and chromosomal constitution appear to be the strongest determinants of morphokinetics and reproductive competence. Integrating these parameters into embryo evaluation frameworks may enhance prognostic accuracy and support more refined selection strategies in ART.

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Ultrafast warming of oocytes and embryos: translational research meets clinical practice

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Aim of the study

To compare a one-step ultrafast warming protocol for vitrified human oocytes and embryos to conventional multi-step warming in terms of survival, developmental competence, and clinical outcomes.

Materials and methods

Three complementary studies were performed:

1. A prospective sibling oocyte study including 818 MII oocytes from 101 oocyte donation cycles. Oocyte survival, fertilization, blastocyst rates and quality were routinely evaluated. Ninety-two single embryo transfers were performed (ultrafast: n=38; standard: n=54), enabling direct comparisons of clinical outcomes between sibling embryos.
2. A retrospective cohort study evaluating 2,677 single frozen embryo transfer (FET) cycles. Blastocysts were warmed using either ultrafast (n=796) or conventional multi-step (n=1,881) protocols. We analyzed biochemical and clinical pregnancy rates following 2,548 single blastocyst transfers (ultrafast: n=719; conventional: n=1,829).
3. A fundamental research study on 225 PGT-A blastocysts cultured to day 10 post-fertilization.

Oocytes and blastocysts were warmed using Kitazato® media, applying either the conventional (1-min thawing solution, TS; 3-mins dilution solution, DS; 5-mins washing solution, WS; transfer to culture media) or ultrafast warming protocol (1-min TS, hold in WS; transfer to culture media).

Results

Ultrafast and conventional protocols achieved comparable survival across all models. In the sibling oocyte study, survival was similar (93.6% vs. 93.3%, p=1.0), while blastocyst rates were significantly higher after ultrafast warming (64.3% vs. 53.7%, p=0.03). Fertilization and clinical outcomes remained equivalent between groups. In the retrospective FET cohort, blastocyst survival was 98.6% (785/796) with ultrafast warming compared to 97.7% (1838/1881) with the conventional protocol (OR=1.66, 95% CI: 0.85-3.24, p=0.138). Biochemical pregnancy rates were modestly higher with ultrafast warming (50.0% vs. 45.4%), both in univariate (p=0.032) and adjusted analyses (OR=1.22, 95% CI: 1.02-1.45, p=0.026). However, clinical pregnancy rates remained comparable (39.9% vs. 37.7%; OR=1.10, 95% CI: 0.92–1.32, p=0.282). Extended culture of PGT-A blastocysts confirmed equivalent blastocyst survival

(98.1% ultrafast vs. 98.3% conventional, p=1.00) and attachment rates. Developmental success was determined by chromosomal constitution rather than warming method.

Conclusion

The one-step ultrafast protocol is a safe, efficient, and clinically validated alternative to conventional warming. By shortening procedure time and reducing variability without compromising results, ultrafast warming offers a practical way to optimize laboratory workflows.

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Embryo cryo protocols in 2025

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Aim of the study

Can an ultrafast vitrification protocol for blastocysts provide comparable or superior survival outcomes to a conventional multi-step vitrification approach?

Introduction

Vitrification protocols traditionally consist of a multi-step vitrification that takes up to 12 min. per embryo. We are currently in the 'fast and the furious' era of cryopreservation (Liebermann J.2025) and colleagues are trying ultra-fast vitrification for embryos (Bosman T. O-120 ESHRE).

Materials and methods

We are also in the process conducting a validation study including a preclinical validation (n = 62 blastocysts) between ultrafast vitrification (n = 16 embryos with artificial collapse (UF+AC) and n=16 without artificial collapse (UF-AC) and the conventional multi-step protocol (MSP) (n =30).

Vitrified surplus blastocysts donated to the biobank for validation purposes were warmed using the single-step protocol (FuijiFilm NX warm kit, 1min 1M trehalose) and re-vitrified according to the MSP or using an ultrafast vitrification protocol (1min ES (100µl) + 45sec VS (100µl) + loading both usingFuijiFilm NX vit kit. After vitrification, blastocyst were rewarmed using the single-step protocol and monitored for 24 hours post-warming in a time-lapse incubator (EmbryoScope™). Intactness (fully – 100% intact) and viability were evaluated using live/dead fluorescent staining to quantify cell damage.

Results

All 62 (100%) blastocysts survived the rapid warming procedure. Fully blastocyst survival was observed in 75% after UF +AC versus 44% after UF -AC compared to 64.3% using MSP. Time-lapse monitoring confirmed a high re-expansion rate with a mean re-expansion time of 3.39 hours in the UF+AC, 5.6 hours in the UF-AC compared to 4.45 hours in MSP. Viability staining showed that a mean cell damage count 6 cells (min: 2 – Max: 18) per blastocyst in UF+AC , 12 cells (min: 1 – Max: 27) per blastocyst in the UF-AC compared 10 cells (min: 0 – Max: 43) per blastocyst in the MSP.

Conclusion

Ultrafast vitrification with AC is beneficial, however we are currently in the process of further validating our protocol with more embryos. Although ultra fast cryopreservation protocols will have an impact on our laboratory workflow, there are still no published clinical application of an ultra-fast vitrification protocol.

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Upublished data, UZGent.

Cryopreservation changes miRNA expression in spermatozoa

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Sperm cryopreservation is routinely used in clinical practice and is widely accepted as a safe and effective procedure. However, it is well-established that this process negatively impacts sperm quality—specifically in terms of viability, motility, and DNA fragmentation. Despite this, there is limited data on how cryopreservation affects epigenetic factors, particularly microRNA (miRNA) expression. miRNAs are crucial post-transcriptional regulators of gene expression, playing an essential role in normal cellular development and function. Importantly, they also contribute to the intergenerational transmission of paternal epigenetic information. Therefore, investigating whether cryopreservation alters sperm miRNA expression is necessary.

To date, only about ten animal studies and five human studies have been published on this topic. These studies consistently show that cryopreservation induces changes in miRNA expression, which can impact fertilization and embryonic development. Moreover, miRNA expression has been found to vary depending on factors such as the duration of cryostorage, the cryopreservation protocol used, and the quality of the native semen sample.

For example, our study demonstrated that both conventional slow freezing and vitrification led to overexpression of selected miRNAs in normozoospermic patients, compared to native semen. In oligozoospermic patients, however, this overexpression was observed only after vitrification. When comparing miRNA expression in native semen samples between normozoospermic and oligozoospermic groups, higher expression levels were found in the latter. Interestingly, after cryopreservation, these differences largely disappeared, suggesting that the process alters the miRNA expression profile of normal semen to resemble that of oligozoospermic samples.

In conclusion, any form of semen cryopreservation alters the miRNA expression profile. Given that these changes may influence fertilization and embryonic development, more comprehensive research is needed to better understand this phenomenon. Such studies could also help identify potential miRNA biomarkers for assessing post-thaw semen quality.

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Epigenomic implication of embryo vitrification – what do we know?

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Embryo vitrification has become the standard method of cryopreservation in assisted reproductive technologies (ART), offering superior post-warming survival compared to slow freezing. Despite its widespread adoption and proven clinical safety, concern remains about potential subtle epigenomic alterations introduced during this procedure. Preimplantation embryos undergo extensive genome-wide reprogramming, leaving them vulnerable to environmental perturbations. The osmotic and thermal stresses of vitrification, as well as cryoprotectant exposure, may influence DNA methylation dynamics, histone modifications, chromatin structure, and non-coding RNA expression.

Evidence from animal studies demonstrates that vitrification can induce locus-specific DNA methylation changes, perturb imprinted gene regulation, and alter placental and offspring gene expression profiles. Some reports link vitrification to differences in neurodevelopmental or metabolic outcomes in animal models, though findings vary by species and protocol. Human studies, largely focusing on placenta and cord blood, identify modest genome-wide methylation differences in ART versus naturally conceived pregnancies, particularly in imprinted regions and pathways related to placental function and angiogenesis. Comparisons of frozen embryo transfer (FET) with fresh transfer cohorts reveal subtle, sometimes sex-specific, methylation signatures. However, effect sizes are small and clinical significance remains uncertain.

Epidemiological studies suggest a slight increase in imprinting disorders such as Beckwith–Wiedemann syndrome in ART-conceived children but disentangling the specific contribution of vitrification from other ART procedures is challenging. The rarity of these conditions underscores that any absolute risk increase remains minimal. Importantly, protocol refinements—such as minimizing cryoprotectant exposure, avoiding repeated freeze–thaw cycles, and optimizing warming steps—likely reduce potential epigenomic stress.

In summary, while animal evidence indicates vitrification can influence embryonic epigenetic programming, human data so far point to subtle and predominantly placental differences with uncertain clinical consequences. Future research should focus on large, well-controlled genome-wide epigenetic and transcriptomic studies that can isolate the specific impact of vitrification, supported by long-term follow-up of offspring health.

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Frozen blastocysts: Day 5 vs day 6 survival and clinical outcomes

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Aim of the study

To determine differences in cryosurvival and pregnancy outcomes when FET cycles are performed with D5 or D6 blastocysts.

Materials and methods

A single center retrospective study was performed from October 2012 till April 2024 including all single FET cycles with D5 or D6 blastocysts.

Results

A total of 1157 FET cycles were included, 1049 with D5 and 108 with D6 blastocysts. Blastocysts were grouped into high quality (HQ) and fair quality group (FQ) according to the blastocyst expansion and morphological grading.

Blastocyst cryosurvival rate (BCR) and live birth rate (LBR) were significantly higher with D5 blastocyst (93.1% vs 83.3%, p=0.0006; 24.3% vs. 14.8%, p=0.035), while percentage of cases of blighted ovum (BO) was significantly higher for D6 blastocysts (4.4% vs 23.1%, p=0.002). However, pregnancy rate (PR) and miscarriage rate (MR) did not differ (29.2% vs 19.1%, p=0.058; 17.6% vs 7.7%, p=0.304). Comparison of D5 and D6 HQ blastocysts showed statistical difference for BCR (92.1% vs. 83.3%, p=0.008), CPR (32.8% vs 20.3%, p=0.033), IR (42.8% vs 29.1%, p=0.027) and BO (5.3% vs 20.8%, p=0.014), but there were no differences among FQ embryos. When separated by type of fertilization, the results showed a higher incidence of male births in the D6 group after ICSI fertilization (20.0% vs 81.8%, p=0.036).

Conclusion

This retrospective study showed significantly higher BCR and LBR for D5 blastocysts, but no other differences in IR and PR were recorded. The cases with blighted ovum were more often recorded in D6 group suggesting that D5 blastocysts is more preferable for ET. Prospective studies with a larger sample size are warranted.

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DET vs. SET: More Is Less!

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In vitro fertilization (IVF) has transformed reproductive medicine, but the choice between double embryo transfer (DET) and single embryo transfer (SET) remains a critical decision impacting maternal and neonatal outcomes. Historically, DET was employed to maximize pregnancy rates, driven by early technological limitations and patient desires for higher success or multiple births. However, advancements in embryo selection, cryopreservation, and clinical protocols have shifted the paradigm toward elective single embryo transfer (eSET). This lecture explores the evidence supporting SET over DET, emphasizing the "more is less" principle. While DET may marginally increase pregnancy rates, it significantly elevates the risk of multiple pregnancies, leading to higher rates of maternal complications (e.g., preeclampsia, gestational diabetes) and neonatal risks (e.g., preterm birth, low birth weight). In contrast, SET offers comparable live birth rates—particularly with high-quality embryos and subsequent frozen cycles—while minimizing these risks. Drawing on clinical guidelines from the American Society for Reproductive Medicine (ASRM), European Society for Reproduction and Human Embryology (ESHRE) and global trends in countries like Sweden, this presentation highlights the safety, efficacy, and ethical advantages of SET. It also addresses patient counseling strategies to navigate preferences for DET and the role of emerging technologies in optimizing SET outcomes. By advocating for SET, clinicians can prioritize healthier singleton pregnancies, demonstrating that transferring fewer embryos yields superior outcomes for both mother and child.

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Recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL) – a view from the IVF lab

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Recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL) are serious problems in IVF/ICSI cycles. Different factors are showed to be responsible for these clinical challenges: paternal, maternal, embryonic, endometrial, immunological and others. Successful implantation depends on the crosstalk between embryo and endometrium. Recurrent implantation failure (RIF) is characterized by a lack of implantation after the transfer of three euploid embryos, occurring in <5% of couples with infertility. It is important to determine the most possible aetiologies, and individualized treatment aimed at the primary cause seems to be an effective method for increasing the implantation rate. The emerging understanding of the biosensor role of the endometrium indicates that, like the embryo, its functional integrity exists on a spectrum rather than being simply receptive or non-receptive. The case is made here that it is the combination of embryo and endometrial quality that determines success, and that the one can compensate in part for the other. Recurrent pregnancy loss (RPL) defined as two or more spontaneous pregnancy terminations occurring before 24 weeks of gestation, affects approximately 1-2% of couples. Altered chromosomal integrity of sperm and oocytes, which is highly dependent of the age of the mother, represents a major cause of miscarriage and in turn RPL. Avoiding transfers of abnormal embryos is possible with preimplantation genetic testing for aneuploidies. In this presentation, we reviewed different possible causes of RIF and RPL and embryological interventions to improve implantation rates and clinical outcomes.

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Evolution of non-invasive preimplantation genetic technique

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In vitro fertilization has helped many couples achieve pregnancy, but significant challenges remain—such as reducing miscarriage rates and improving overall success. One of the key steps is selecting a healthy, genetically normal embryo for transfer. Preimplantation genetic testing was introduced as a tool for analyzing the embryo's genetic material before implantation, relying on an invasive trophectoderm (TE) biopsy.

This seminar will explore the evolution of non-invasive preimplantation genetic testing (ni-PGT) and minimally invasive preimplantation genetic testing (miPGT), which analyses cell-free DNA (cf-DNA) from embryo culture media or blastocyst fluid. We will trace the key technological advancements that have overcome initial challenges to improve its accuracy and reliability. The discussion will cover clinical data, comparing the utility and limitations of niPGT with the traditional biopsy.

We will conclude by looking ahead at how this non-invasive approach may revolutionize embryo selection by reducing stress on the embryo and expanding access to genetic screening in assisted reproduction.

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ICSI for patients with non-male factor infertility

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Intracytoplasmic sperm injection (ICSI), introduced in 1992 as an alternative of in vitro fertilization (IVF), was originally indicated for male factor infertility or fertilization failure. Its use has since expanded significantly, including in couples without male factor infertility, though evidence supporting such practice remains limited. This presentation synthesizes current data on ICSI in non-male factor infertility regarding efficacy, safety and cost-effectiveness.

ICSI utilization has risen from ~20% of IVF cycles to >70%, with the largest increase in non-male factor cases (15% to >60%). However, large cohort studies and registry analyses show no improvement in cumulative live-birth rates compared to conventional IVF in this population, despite reduced rates of fertilization failure.

Proposed indications include unexplained infertility, low oocyte yield, poor-quality oocytes, advanced maternal age, prior fertilization failure, preimplantation genetic testing (PGT), in vitro maturation (IVM) and use with cryopreserved oocytes. Evidence indicates:

- **Unexplained infertility:** ICSI lowers fertilization failure but does not increase live-birth rates.
- **Low oocyte yield / poor-quality oocytes / advanced maternal age:** no demonstrated benefit in fertilization, embryo quality, pregnancy, or live-birth outcomes.
- **Prior fertilization failure:** ICSI significantly improves fertilization and is justified.
- **PGT:** required only when sperm contamination would compromise genetic testing; otherwise not necessary.
- **IVM oocytes:** ICSI improves fertilization, but implantation and pregnancy may be higher with conventional IVF.
- **Cryopreserved oocytes:** ICSI is the preferred method due to zona pellucida changes post-thaw.

Safety concerns remain, as ICSI involves more manipulation, cost and time. Large population studies suggest a slightly increased risk of congenital anomalies, though confounding by paternal factors is possible. Cost-effectiveness analyses indicate >30 unnecessary ICSI cycles are required to prevent one case of unexpected fertilization failure.

Conclusion

ICSI is indispensable for male factor infertility and select non-male factor scenarios - prior fertilization failure, cryopreserved oocytes and PGT where contamination risk exists. In other contexts, while it may reduce fertilization failure, it does not improve live-birth rates and adds cost and risk. Current evidence and professional guidelines do not support its routine use in non-male factor infertility; decisions should be individualized based on clinical history and laboratory expertise. Further prospective studies are needed to clarify long-term outcomes.

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Is microfluidic sperm selection the best option for daily lab routine?

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Aim of the study

Human reproductive treatments still demonstrate limited efficiency, averaging only ~33% success per cycle, despite many technical improvements and add-ons in ART. This persistent limitation has stimulated a search for advanced approaches to sperm preparation for ICSI. Among new strategies, microfluidic sperm separation (MSS) has gained strong attention and is now considered one of the most promising tools.

Conventional methods such as swim-up (SU) and density gradient centrifugation (DGC) depend mainly on motility, count, and morphology. Yet these parameters only partly reflect fertilization competence. For embryologists, the daily task is to isolate sperm with higher DNA integrity, progressive motility, and optimal morphology. Research increasingly suggests that MSS enriches such populations, improving laboratory outcomes, though clinical evidence is still limited.

Aim of this study is to evaluate the effect of MSS on clinical pregnancy rate (CPR) in our hospital and to compare fertilization and blastocyst rates between MSS and conventional sperm preparation.

Materials and Methods

From Sept 2024 to July 2025, 1,031 couples-initiated treatment, and 128 selected MSS (ZyMot). Controls underwent DGC. Exclusions included OPU without eggs, TFF, and donor gametes. Cut-off values: semen concentration $\leq 3x10^6$/ml and progressive motility $<0.1x10^6$/ml. Data were analyzed according to female age (<40 and ≥40 years).

Results

In women <40, MSS cycles showed a modest fertilization increase (3.69%), while in ≥40 the rise was substantial (9.18%). Blastocyst rates were comparable. Clinical pregnancies were confirmed by ultrasound at 7 weeks. In <40, CPR increased from 36.42% with DGC to 46.34% with MSS, though no overall benefit appeared in ≥40. Subgroup analysis by ET day revealed striking improvements. For day 3 ET in <40, CPR rose from 26.77% to 40.63%. For day 5 ET, results were stronger: in <40, CPR jumped from 49.32% to 66.67%, and in ≥40 from 23.81% to 50.00%. The average embryos per ET were similar.

Conclusion

Despite limitations, such as poor baseline sperm quality and absence of miscarriage or live birth data, our study indicates that MSS enhances CPR, especially in day 5 transfers and in older patients. With improved DNA integrity, reduced handling time, lower device cost, and strong automation potential, MSS is well positioned to become the preferred method for routine sperm preparation in ART practice.

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Quality control in the IVF laboratory

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Quality control (QC) in the in vitro fertilization (IVF) laboratory is essential for safety, reproducibility, and optimal patient outcomes. IVF success is influenced by more than 200 biological and technical factors, and small deviations can compromise gamete and embryo viability. Effective QC must therefore stabilize the laboratory environment, validate processes, and document deviations within a robust quality management system (QMS).

Fundamental QC measures, include strict control of incubator temperature, pH, osmolality, and gas composition. Even minor fluctuations—such as temperature drops during oocyte aspiration, evaporative shifts when dish lids are left open, or CO_2 calibration errors—directly affect embryo development. Consumables and media pose further risks due to lot-to-lot variation, packaging-derived toxins, and volatile organic compounds (VOCs). Air quality, often underestimated, is equally critical for embryo viability. Staff diligence, adherence to SOPs, and emergency preparedness, including cryostorage contingency planning, are indispensable pillars of laboratory stability.

While these fundamentals are clear, real-world practice reveals heterogeneity. Palmer and colleagues (2019) conducted a multinational study of 36 IVF laboratories using a cloud-based QC platform. Incubators accounted for 35% of all monitored parameters, yet discrepancies between displayed and measured values were frequent. Cryostorage monitoring was inconsistent, and VOC surveillance often absent. The study introduced the Mean Average Data (MAD) score as a surrogate for QC diligence, finding higher scores in countries with regulation and mandatory audits. Their findings illustrate that electronic tools enhance monitoring, but diligence and standardization remain essential.

Complementing these findings, Hammond and Morbeck (2019) demonstrated that laboratory performance can be tracked statistically through key performance indicators (KPIs). They showed that the Day 5 usable blastocyst rate (D5BUR) was a more sensitive KPI than overall blastocyst rate or clinical pregnancy rate. In their study, D5BUR detected shifts in laboratory performance months earlier than clinical outcomes, providing an early-warning signal for corrective action. This reinforces the importance of embedding KPI surveillance into routine QC programs.

Together, these studies underscore a dual reality: IVF laboratories understand the critical variables, but application of QC remains inconsistent, and clinical outcomes are slow to reveal deficiencies. Bridging this gap requires both diligence and innovation. Diligence means proactive, disciplined, and consistent monitoring, supported by corrective and preventive actions (CAPA). Innovation includes adoption of cloud-based QC platforms, electronic witnessing, RFID traceability, and AI-assisted embryo assessment. Cost-effectiveness analyses suggest these investments reduce error-related costs, improve staff efficiency, and strengthen compliance ultimately delivering clinical and financial value.

In conclusion, quality control in IVF is both a science and a culture. Fundamentals such as temperature, media, and air quality remain non-negotiable. Evidence from global audits demonstrates the need for standardization, while KPI-driven surveillance provides sensitive markers of performance. Advanced digital tools support, but do not replace, the diligence of embryologists. The IVF laboratory of the future will combine rigorous fundamentals with electronic oversight and AI-driven insight, ensuring safety, stability, and reproducibility for patients worldwide.

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The better mentor...The better mentee

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Aim of the study

Education of the embryologist is the main and critical part of the quality and safety in the ART procedures. This lecture is a proposal for the standardization of training and education of clinical embryologists in Croatia.

Materials and methods

The literature guidelines regarding clinical embryologists training and education from Regulation of the European Parliament, European Directorate for the Quality of Medicines and HealthCare, EDQM, European Society of Human Reproduction and Embryology ESHRE and ALPHA Scientists in Reproductive Medicine were compared with Croatian national legislation and guidelines of the Croatian society of the clinical embryologists (HDKE).

Results

Data from the literature for the countries of the European Union showed that only 10 out of 31 countries have a prescribed minimum qualification and education for clinical embryologists in their national documents. Mandatory education was reported by 9 countries, but only 4 of them have organized postgraduate education or training for independent work in IVF laboratory. Recognition of the „clinical embryologist" profession is only 35%. Predominant education is "informal" education.

European Union of medical specialist - UEMS suggested that organized education should include prescribed theoretical knowledge (curriculum), a training program (syllabus), a tutorial system with prescribed criteria for tutors, a logbook, an independent system for checking theoretical knowledge and practical skills, competence assessment once trained, continuing professional development CPD. Part of the Croatian legislation and the HDKE educational program contain some similar parts, but in Croatia we need to standardize the education model through professional education and legislation.

In the model of "informal" education the key role has mentor and his competences. In Croatia, the criteria for mentoring are based solely on years of work experience. A prescribed criteria for mentors is still missing, and here we try to define some minimal standards.

Conclusion

According to the UEMS standards, Croatian Society of the Clinical Embryologist have established all necessary steps for the professional education and master training of biologists for the position of clinical embryologists. It is necessary to prescribe strict criteria for the mentors. The goal is to move from "informal" to a higher level of education for the profession of clinical embryologist in Croatia.

Key references:

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The New EU SoHO Regulation: Implications for MAR Practice

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Aim of the study

This work aims to provide an overview of the new EU Regulation (2024/1938) on Substances of Human Origin (SoHO) and its implications for Medically Assisted Reproduction (MAR), highlighting key differences from the EU Tissues and Cells Directive (2004/23/EC) and future requirements for clinics and regulators.

Materials and methods

The abstract is based on a comparative review of EU legislative documents (Directive 2004/23/EC, Regulation 2024/1938), technical guidance from the European Directorate for the Quality of Medicines and Healthcare (EDQM) and the European Centre for Disease Prevention and Control (ECDC), as well as national transposition requirements relevant to Croatia.

Results

The SoHO Regulation, effective from August 2024 with implementation by August 2027, establishes a unified legal framework for all substances of human origin, including gametes and embryos, under the concept of "reproductive SoHO." Unlike the Directive, the Regulation is directly applicable in all Member States, ensuring greater harmonization. It introduces stricter provisions for donor, recipient, and offspring protection, mandatory quality management systems, accreditation of SoHO entities, and regular inspections of MAR laboratories and gamete banks. New elements include the SoHO Coordination Board (SCB), an EU-wide platform for vigilance and reporting, and annual activity reports. Traceability of SoHO is extended to 30 years, with additional vigilance obligations for third-party donor complications (e.g., severe OHSS).

Conclusion

The transition from Directive to Regulation represents a paradigm shift for MAR practices across Europe. Harmonization, transparency, and strengthened donor and recipient safeguards will increase accountability of IVF laboratories and national authorities. Early preparation of clinics for accreditation, digital reporting, and compliance with EDQM/ECDC guidelines is essential ahead of the 2027 deadline.

Key references:

Directive 2004/23/EC of the European Parliament and of the Council on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.

Regulation (EU) 2024/1938 of the European Parliament and of the Council on standards of quality and safety for substances of human origin intended for human application.

European Directorate for the Quality of Medicines & HealthCare (EDQM), Guide to the quality and safety of tissues and cells for human application, latest edition.

Data transfer – to whom, when, why?

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This presentation will contain information on the different levels of data registries that are used or in the making: national registries, European registry (EuMAR) and the new activity-based registry on the (to be developed) SOHO platform. National registries are obligatory, as well the future SOHO platform. EuMAR on the other hand is a voluntary-based register. Also the type of data that is collected in these platforms is different: some is cycle-by-cycle, others are summary data (table 1).

Feature	EuMAR Registry	EU SoHO Platform	Croatian IVF Registry	Belgian IVF Registry (BELRAP)
Focus	MAR treatment outcomes	Regulatory compliance for SoHO	Legal access and clinical practices	National MAR treatment monitoring
Managed by	ESHRE	European Commission	Croatian Ministry of Health	College of Physicians of Reproductive Medicine
Data Type	Cycle-by-cycle clinical data	Summary activity data	Clinic-level data	Cycle-specific clinical data
Submission	Voluntary (may become mandatory)	Mandatory	Clinic-dependent	Mandatory for ART centres
Scope	IVF, ICSI, IUI, fertility preservation	All SoHO activities	IVF, IUI, embryo donation, PGT	IVF, ICSI, thawed cycles, outcomes
Traceability	IRCC-based	30-year traceability with EU codes	Limited	Unique BELRAP ID per cycle

This talk will try to show you the connection between the different registries or the lack thereof. Finally, a sneak preview of the data (probably) to be collected in the SOHO platform will be shown.

POSTERI / POSTERS

The Effect of Combined Oral Contraceptives Compared with Metformin on Acne and Hyperandrogenism in Women with PCOS Phenotype A

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in reproductive-age women, often presenting with acne and biochemical hyperandrogenism. Guidelines recommend combined oral contraceptives (COCs) as first-line therapy for menstrual irregularities and hyperandrogenism, while metformin is intended for women with insulin resistance (IR) or metabolic disturbances. Despite this, it is frequently prescribed outside these indications, largely due to concerns about hormonal contraceptive side effects.

Objective

This retrospective study compared the effects of COCs and metformin on acne and biochemical hyperandrogenism in women with PCOS phenotype A, with additional subgroup analysis by baseline insulin resistance and overweight.

Materials and Methods

Records of 50 women aged 18–35 years with PCOS phenotype A (Rotterdam criteria) were reviewed. All had acne and elevated testosterone. Patients were grouped as COC (ethinylestradiol 30 µg + drospirenone 3 mg, n=25) or metformin (1500 mg/day, n=25). Subgroups were defined by baseline IR (HOMA-IR >2.5) and/or BMI ≥25 kg/m^2. Data included acne severity (GAGS), hormonal profile, and metabolic measures at baseline and six months.

Statistical Analysis

Within- and between-group comparisons were made using standard parametric or non-parametric tests; categorical data with chi-square/Fisher's exact test. Significance was set at $p<0.05$.

Results

Forty-seven women completed follow-up (24 COC, 23 metformin). Acne improved in both groups, but reduction was greater with COC (–56%, 18.2->8.1) than metformin (–27%, 17.9->13.0; $p<0.01$). COCs also lowered total testosterone (2.6->1.8 nmol/L), reduced free androgen index (7.8->2.5), and increased SHBG (34->78 nmol/L; $p<0.001$). Metformin showed little hormonal effect overall but in women with IR/overweight (n=14) HOMA-IR declined from 3.0->2.1 (–30%), and BMI from 28.7->27.2 ($p<0.05$). No significant metabolic changes occurred in metabolically healthy women (n=11). COC users showed stable metabolic parameters. Adverse events included gastrointestinal symptoms in six metformin users (two discontinued) and mastalgia/headache in two COC users (one discontinued).

Conclusion

COCs were more effective in improving acne and hyperandrogenism, while metformin provided metabolic benefits mainly in women with insulin resistance or excess weight. These findings support COCs as first-line treatment for hyperandrogenism, with metformin reserved for patients with metabolic risk. An individualized approach remains essential, and combined therapy may offer complementary effects.

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Cryopreservation as a Method of Fertility Preservation in a Woman with Breast Cancer – A Case Report

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Aim

To highlight the importance of fertility preservation in young patients with invasive breast cancer. Breast cancer is one of the most common malignant tumors in women and the leading cause of cancer-related death. In 2022, it caused 670,000 deaths worldwide. Although it is more frequent after menopause, there is a rising incidence among women younger than 40, particularly in the 20–29 age group. In this population, more aggressive subtypes are common, with higher rates of metastasis and mortality, making these patients candidates for aggressive chemotherapy. Chemotherapy often causes gonadotoxicity and may result in permanent ovarian damage, underlining the importance of fertility preservation.

Materials and methods

Presentation of a multidisciplinary approach to fertility preservation in a young woman diagnosed with invasive breast cancer.

Result

The case involves a 34-year-old nulliparous patient who was diagnosed with invasive breast cancer during an assisted reproduction procedure. The patient initially underwent tumor excision, followed by a more radical procedure consisting of quadrantectomy and axillary clearance. Due to residual positive tumor tissue and micrometastases in two lymph nodes, a right mastectomy was ultimately performed. Pathohistological analysis confirmed luminal B type carcinoma: ER +++, PR +++, Ki-67 index 23.17%, and HER2 negative. At the multidisciplinary oncology tumor board, the decision was made to proceed with adjuvant chemotherapy, radiotherapy, and hormone therapy. Before adjuvant treatment, the patient was referred to a gynecologist for fertility preservation consultation. Her serum anti-Müllerian hormone (AMH) level was 17.19 µg/L, compared to 37.74 µg/L three years earlier. Five weeks after mastectomy, she underwent the necessary evaluation, followed by ovarian stimulation and embryo cryopreservation prior to adjuvant oncological treatment. Over the next five years, she underwent extensive oncological therapy, including 12 cycles of adjuvant chemotherapy according to the ACT DD protocol, 19 fractions of radiotherapy, and hormone therapy (LHRH agonist – leuprorelin, and an aromatase inhibitor). Two years after completion of treatment, with normal follow-up findings from oncology, radiology, and endocrinology, the patient was advised to plan pregnancy. At the age of 41, through embryo transfer, she achieved a biamniotic, bichorionic twin pregnancy, which progressed normally and was delivered by cesarean section at 37 weeks, resulting in two live, term, eutrophic infants.

Conclusion

Embryo and oocyte cryopreservation are the most common fertility preservation methods. Ovarian tissue cryopreservation is possible but limited - especially in women with BRCA1/2 mutations due to the increased risk of ovarian cancer. According to ASCO guidelines, all women of reproductive age should be informed about the risk of infertility and referred for counseling. An individualized

approach is necessary. The increasing number of young women with breast cancer and longer survival rates make fertility preservation an essential part of oncological care. The choice of method depends on age, tumor type, genetic profile, and the time available before treatment initiation.

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Ahmad MF, Sugishita Y, Suzuki-Takahashi Y, Sawada S, Iwahata H, Shiraishi E, Takae S, Horage-Okutsu Y, Suzuki N. Young adults with breast cancer: a case series of fertility preservation management and literature review. Front Med (Lausanne). 2021 Aug 6;8:670872.

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Incidence and Perinatal Outcome of Pregnancies in Women Aged Over 40 in a One-Year Period in KBC Split

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Aim

The aim of this study was to examine the incidence of pregnancies in women older than 40 years and to analyze perinatal outcomes over a one-year period at the University Hospital Center Split. Considering the increasing trend of delaying motherhood, pregnancies in later life and reproductive age are becoming more frequent, but they are accompanied by increased risks for both mother and fetus, including gestational diabetes, hypertension, preterm birth, and a higher likelihood of operative delivery.

Materials and methods

The study was conducted as a retrospective cross-sectional study on a sample of 197 pregnant women aged 40 years and older who gave birth at UHC Split between January 1st and December 31st, 2024. The analysis included demographic data, obstetric history, presence of complications, mode of delivery, and perinatal outcome.

Results

During the analyzed period, there were 4185 deliveries. Of these, 197 were women older than 40 years (4.7%). A total of 24 pregnancies (12.2%) were achieved using assisted reproductive techniques (ART), 15 among primiparas (7.6%) and 9 among multiparas (4.5%). Among them, three women had twin pregnancies (two dichorionic, one monochorionic), all of which resulted from ART and were delivered prematurely by cesarean section. A body mass index (BMI) greater than 30 was recorded in 70 women (35.5%), while 104 women (52.8%) had a BMI between 25–30. In the group of women with a BMI over 30, significantly more primiparas delivered by cesarean section compared to multiparas, $p=0.00075$. Gestational diabetes was diagnosed in 23 women (11.7%): 11 with a BMI over 30, 9 with a BMI between 25–30, and only 3 with a normal BMI. There were 8 preterm births (4%). A total of 38 newborns (19.3%) were macrosomic. The overall cesarean section rate was 45.7%, while vaginal deliveries accounted for 54.3%. Among the 40 primiparas who conceived spontaneously, 4 delivered vaginally and 21 by cesarean section, while among those who conceived via ART, 1 delivered vaginally and 14 by cesarean section. There was no statistically significant difference in delivery mode between spontaneous and ART pregnancies in primiparas, $p>0.05$. Among 157 multiparas who conceived spontaneously, 100 delivered vaginally and 48 by cesarean section, while among ART pregnancies, 2 delivered vaginally and 7 by cesarean section, which represents a statistically significant difference in favor of vaginal delivery in spontaneous pregnancies, $p<0.01$. There were more primiparas in the ART group (7.6% vs. 4.5%). The oldest primipara who conceived through ART was 49 years old, and the pregnancy was completed at 38 weeks by cesarean section. Perinatal outcomes assessed by Apgar score were 10 in 87% of cases. There was one case of stillbirth at 26 weeks from a spontaneous third pregnancy, delivered vaginally.

Conclusion

Pregnancy at age over 40 represents a significant clinical challenge. An individualized approach and close monitoring of risk factors are essential in order to detect and prevent adverse events as early as possible. With quality prenatal care, the majority of these pregnancies can have favorable outcomes.

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The importance of laparoscopy in tubal infertility factor

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Introduction

According to the World Health Organization (WHO), infertility is defined as a couple when, despite regular unprotected intercourse, there is no pregnancy within one year. Pathology of the tubes is the most common reason for infertility in a woman. Silent and repeated inflammations significantly more often damage the distal end of the tube (85% of cases) than the proximal one. Only laparoscopy, with possible tuboscopy, can assess the degree of damage to the fallopian tubes, the extent of adhesions and the decylation of endosalpinx. HSG is a method that examines the condition of the cervical canal, the shape and size of the cavity, the position of the uterus, the patency of the tubes, anomalies and diseases of the tubes, as well as the relationship of the uterus and tubes with the surrounding organs. Laparoscopy, or minimally invasive surgery, is a form of modern surgery in which surgery in the abdominal cavity is performed through small puncture wounds on the anterior abdominal wall.

Aim of this study is to evaluate the success of laparoscopic salpingostomy in the treatment of hydrosalpinx, i.e. Terminal tube occlusion in infertile women based on the rate of patency of the operated tube 3 months after surgery, as assessed by hysterosalpingography.

Material and methods

This prospective study included 137 patients diagnosed with primary or secondary infertility. The research was conducted in the GAK Narodni Front in the period from 2020. to the end of 2021. The first subgroup consisted of 66 patients with primary infertility and patients with secondary infertility. All subjects had a specific test protocol. In the study of tubal infertility factor, hysterosalpingography was first performed, and then laparoscopy. Follow-up hysterosalpingography was performed after 3 months. The aim of this study is to evaluate the success of laparoscopic salpingostomy in the treatment of hydrosalpinx, i.e. terminal tube occlusion in infertile women, based on the rate of patency of the operated tube 3 months after surgery, which was assessed by performing hysterosalpingography.

Discussion

There is a significant difference in the distribution of subjects according to the findings of ultrasound before surgery. Namely, among pathological ultrasound findings, the existence of unilateral hydrosalpinx is the most common, while the association of unilateral hydrosalpinx and fibroids was the rarest pathological ultrasound findings. Also, the finding without pathological changes was the most common finding. Among the pathological findings, terminal obturation of the right fallopian tube is the most common, while bilateral terminal obturation of the jaws is the rarest pathological HSG finding. In patients with primary infertility, terminal obturations of the right fallopian tubes are also the most common, and bilateral terminal obturations of the jaws are the rarest pathological HSG findings. Among the pathological findings on the fallopian tubes, hydrosalpinx of the left fallopian tube is the most common, while bilateral terminal obturation of the fallopian tubes is the rarest pathological laparoscopic finding. In subjects who underwent control HSG 3 months after laparoscopic surgery, no significant difference was found in pathological findings on the fallopian tubes.

Conclusion

However, a significant difference was found in the distribution of normal findings on the control HSG. Namely, the most common normal finding is the normal patency of the right fallopian tube, while the patency of both fallopian tubes was the least represented.

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Effect of cytokines on endometrial receptivity

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Introduction

An endometrial polyp is most often a benign localized proliferation of endometrial glands and stroma covered with epithelium above the plane of the mucosa. They are most often diagnosed during examination of the cause of irregular uterine bleeding or infertility. Glycodeline is a 28 kDa glycoprotein containing 180 amino acids. Glycodeline is found in the glandular and superficial epithelium of the endometrium. It is produced in the highest concentration during the secretory phase of the endometrial cycle. The lowest concentrations are in the follicular phase. Glycodeline levels peak 12 days after ovulation.

INF γ is the most important pro-inflammatory cytokine responsible for activating and regulating the phagocytic function of mononuclear cells. It is produced by activated T lymphocytes and T4 lymphocytes in response to IL-12, IL-18, NK cells, monocytes, and macrophages. It plays an important role in the control of bacterial, viral and parasitic infections. It can be considered the main effector cytokine in the immune response. INF γ secreted primarily by Th lymphocytes (T helper), CTL (cytotoxic T lymphocytes), NK cells and macrophages in response to IL-2, mitogens, antigens or the associated effects of IL-12 and IL-18.

Aim

The aim of this study is to present the concentration of Glycodeline I INF γ, obtained by the ELISA method, before and after hysteroscopic polypectomy in infertile patients in serum and uterine lavage, as well as to experiment with Glycodelin, INF γ obtained by immunohistochemical method in endometrial biopsy and polyp tissue.

Material and methods

The study included 82 infertile patients. The first group was designated as an experimental group and consisted of 56 infertile patients with endometrial polyps. The second group was designated as a control group and consisted of 26 infertile patients without endometrial polyps.

Discussion

The obtained results primarily indicate the existence of changes in the expression and concentration of Glycodeline I INF γ in experimental and control patients, both before hysteroscopy and after hysteroscopy.

Conclusion

Endometrial polyp can be one of the causes of female infertility.

In patients of the experimental group, the serum cytokine Glycodeline I INF-γ may be a screening test for the detection of endometrial polyps.

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Analysis of genetic factors of coagulation in infertile patients

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Objectives

The aim of our study was to assess the frequency of mutations of inherited blood clotting factors in patients treated for infertility, as well as the association of these mutations with the duration of the infertility treatment.

Materials and methods

This study included 493 patients who were treated for infertility at the Department of Human Reproduction at the Clinical Hospital Center Rijeka, all of whom became pregnant after the treatment. Patients were tested for mutations in blood clotting factors (plasminogen activator inhibitor-1, methylenetetrahydrofolate reductase, factor II and factor V Leiden). A total of 114 subjects had a proven mutation in at least one of the tested genes, while 379 patients had no proven mutation. Among the patients with a mutation, 60 patients were homozygous and 54 were heterozygous for at least one of the proven mutations. The treatment duration and the number of assisted reproductive technology cycles until conception were assessed for each patient. The control group consisted of patients without a proven mutation.

Results

Among the patients with a proven homozygous mutation, the mean time until conception was 13.58 months (p=0.0003), and among the patients with heterozygous mutation the mean time until conception was 12.28 months (p=0.03). Comparatively, among the patients in the control group conception occurred after a mean interval of 7.79 months. Similarly, patients with a homozygous mutation required a mean of 4.18 cycles of treatment until conception (p=0.002), while patients with heterozygous mutations required a mean of 4.04 cycles until conception (p=0.02) and patients in control group required 3.19 cycles until conception. When we compared patients with homozygous and heterozygous mutations, there was no statistically significant difference in both duration of the treatment and number of required ART cycles until conception.

Conclusion

Mutation of one of the blood clotting factors can be associated with longer infertility treatment and a higher number of ART cycles until conception. Our results prove that presence of the mutation significantly affects the success of the treatment, not the type of mutation.

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EUmetriosis - "Transforming endometriosis care in Europe via an integrated approach addressing current knowledge, diagnosis, tailored management and patient empowerment"

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Endometriosis is a chronic inflammatory disease characterized by the presence of endometrial-like tissue outside the uterus, affecting approximately 6-10% of women. Symptoms include dysmenorrhea, dyspareunia, pelvic pain, gastrointestinal and urinary symptoms but also infertility, which all can negatively affect quality of life. Women with endometriosis experience 8-10 years of delay in the diagnosis from the onset of their symptoms before receiving adequate care and therapy. Treatment options typically include hormone therapies and/or surgery. Hormone therapies are not curative, can have significant side effects and are contraceptive. The aim of surgery is to remove endometriotic lesions, but patient can have recurrence in up to 50% within 5 years of surgery. Many patients seek advice regarding lifestyle changes, such as dietary adjustments or exercise, but research on the effectiveness of these approaches remains limited. There is a need for early diagnosis and more effective pain management for women who live with endometriosis burden.

A consortium of partners led by Prof. MM Dolmans from Belgium has launched an EU-funded project (Horizon): "Transforming endometriosis care in Europe via an integrated approach addressing current knowledge, diagnosis, tailored management and patient empowerment" in short "EUmetriosis".

Each partner is focused on a different aspect of endometriosis research, and we, in collaboration with our UK partners at the University of Edinburgh led by prof A. Horne plan to enroll a total of 500 patients (250 from the UK and 250 from Croatia) who have been diagnosed with endometriosis (by ultrasound or surgery). With the support of UK and Croatian endometriosis patient organizations, we will advertise the study through social media and other online means of recruiting researchers, as well as by verbally informing patients about the study during their gynecological check-ups. Patient recruitment will last 24 months. Our goal to accelerate discovery and advance data-driven research into endometriosis diagnosis and treatment by collecting large, multimodal, longitudinal data. Our study is a longitudinal cohort study, which will give us the ability to identify and relate changes in symptoms due to medical, surgical, and self-management, and to correlate these

changes with patterns in the gut microbiome and blood metabolome. Our chosen study design will also allow us to further define these exposures with regards to presence, timing and chronicity and follow change over time in particular individuals within the cohort.

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Peritoneal Inclusion Cysts in an Adolescent Patient Without Risk Factors – A Diagnostic Challenge and the Importance of 3D Ultrasound

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Introduction

Peritoneal inclusion cysts are rare but clinically significant formations that may present as adnexal masses in adolescent patients. Differential diagnosis often includes ovarian neoplasms, which may lead to invasive diagnostic procedures. Ultrasound diagnostics are essential in recognizing these formations, especially in younger patients, thereby reducing the need for surgical interventions.

Case Presentation

An adolescent patient was initially referred for internal medicine evaluation due to progressive hair loss. Comprehensive evaluation did not identify a clear cause of symptoms. During the diagnostic process, an abdominal ultrasound revealed free peritoneal fluid with thin septa. Abdominal MRI confirmed these findings, leading to a referral for gynecological evaluation. The patient was a virgin, with no history of abdominal surgeries, inflammatory bowel disease, or prior gynecological conditions that could predispose the development of peritoneal inclusion cysts. Gynecological ultrasound at a referral center provided key information for diagnosis. A detailed transrectal 3D ultrasound revealed the presence of multiple septated cystic formations located peri-adnexally, which did not show signs of compression on surrounding structures. Ovarian parenchyma was normal, without solid masses. The findings were consistent with peritoneal inclusion cysts, a rare diagnosis in adolescence. Laboratory tests showed mildly elevated CA-125 levels. During follow-up, CA-125 levels showed a declining trend. Given the normal hormonal status, preserved ovarian structure, and stable findings, a conservative management approach was agreed upon with the patient, involving regular clinical and ultrasound monitoring, as well as tumor marker evaluation to exclude future progression. After twelve months of follow-up, no progression of cystic formations or increase in tumor marker levels was observed.

Discussion

Peritoneal inclusion cysts represent a diagnostic challenge in the differential diagnosis of adnexal masses in adolescents. They are often secondary to intra-abdominal adhesions but can also arise without a clear predisposing factor. A key ultrasound finding is the presence of septated fluid-filled spaces that follow the contours of adjacent structures without a mass exerting pressure. Differential diagnoses include ovarian cysts, endometriomas, and tumors. Ultrasound helps distinguish these entities, as peritoneal inclusion cysts lack vascularization and do not affect neighboring structures. Proper interpretation of ultrasound findings reduces the need for invasive procedures and allows safe conservative management.

Conclusion

Ultrasound remains the primary imaging method for evaluating adnexal masses in adolescent patients. Recognition of the characteristic ultrasound features of peritoneal inclusion cysts significantly contributes to optimizing clinical management and avoiding invasive procedures. In this

case, the novel 3D ultrasound technology played a crucial role in establishing the diagnosis, enabling precise visualization of cystic structures and supporting a safe conservative treatment approach.

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Primary Vaginal Fibroma – A Diagnostic Challenge – A New Possibility of 3D Technology

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Introduction

Vaginal fibromas, histologically also known as leiomyomas, are rare benign smooth muscle tumors, frequently described in the literature as extremely rare neoplasms of the female genital tract. They are most located on the anterior vaginal wall, while cases localized on the lateral and posterior vaginal walls are significantly less frequent. Clinically, they may present with a wide spectrum of symptoms, including dyspareunia, a sensation of pressure, walking discomfort, dysuria, constipation, vaginal mass, and less commonly, vaginal bleeding. The diagnostic approach involves bimanual examination and imaging methods, with transvaginal ultrasound (US) and magnetic resonance imaging (MRI) being the main modalities of choice. MRI provides superior soft tissue contrast resolution and is used in cases requiring detailed evaluation of tumor relationships with adjacent structures. However, ultrasound remains the first-line method due to its wide availability, non-invasiveness, cost-effectiveness, and real-time dynamic assessment. Three-dimensional ultrasound (3D US) further enhances diagnostic accuracy by providing more detailed visualization and precise tumor localization, enabling reliable and timely diagnosis even in complex cases.

Case Presentation

A 65-year-old patient was referred to a gynecology clinic due to the presence of a tumor mass in the vaginal region. She had no significant gynecological complaints in the past, her menstrual history was unremarkable, and there were no records of previous gynecological procedures. On examination, a firm, painless mass approximately 6 cm in diameter was identified on the anterior vaginal wall, without signs of inflammation or mucosal ulceration. An aspiration was initially performed to evaluate the nature of the lesion, yielding mucinous content. This finding was nonspecific and did not allow for a definitive diagnosis. Due to the tumor's anatomical position and possible communication with the bladder, MSCT urography was indicated. The findings did not provide a conclusive answer regarding the presence of a fistulous connection between the tumor and the bladder, leaving the possibility open. To further clarify the tumor's relation to the bladder, a three-dimensional transvaginal ultrasound was performed, which enabled precise visualization. The tumor was clearly identified as a separate formation in front of the vagina, distinct from the bladder, thus ruling out communication. Tumor markers were also determined and found to be within normal range, excluding malignant processes. Given the symptomatic nature of the tumor and its clear separation from other pelvic organs, surgical removal was performed via a vaginal approach. Histological analysis confirmed a benign vaginal leiomyoma without atypia or malignancy.

Discussion

Vaginal fibromas are extremely rare benign lesions, with a prevalence significantly lower than uterine leiomyomas. They are most located on the anterior vaginal wall, while lateral and posterior localizations are rarer. Due to their rarity, they are often misdiagnosed as Bartholin's cysts, urethral diverticula, or vaginal metastases of other tumors. In larger tumors, compression of adjacent organs may occur, leading to symptoms such as dyspareunia, pelvic pressure, dysuria, or constipation. Standard diagnostics include bimanual examination and transvaginal ultrasound, but in cases where more precise localization of the tumor is required, three-dimensional ultrasound proves to be an

indispensable tool. In this case, MSCT urography failed to clearly define the tumor's relationship with the bladder, while three-dimensional ultrasound provided definitive confirmation of anatomical separation, ensuring optimal surgical planning and reducing the risk of intraoperative complications. Surgical removal is the treatment of choice, with a vaginal approach often preferred due to minimal invasiveness and rapid recovery.

Conclusion

Although MRI may be useful in complex cases, 3D ultrasound has proven to be a cost-effective, widely available, and dynamic method that in many situations provides equally precise information as MRI. The non-invasiveness of 3D ultrasound, its real-time assessment capability, and precise visualization of tumor relationships with surrounding structures enable quick and effective clinical decision-making. Given the rarity of this pathology, it is necessary to raise clinicians' awareness of the importance of accurate imaging diagnostics to ensure timely diagnosis and optimal treatment while avoiding invasive procedures and unnecessary costs.

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The role of 3D ultrasound in the diagnosis of a forgotten rare intrauterine device

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Introduction

The intrauterine device (IUD) is an effective, long-lasting and reversible form of contraception. Various forms of intrauterine devices have been used for the past hundred years, and since the 1960s, the so-called spiral (Beospir) has been used, which got its name because of its coil-like shape. In the last century, intrauterine devices were placed at an early age and were not changed until menopause, or even later. For this reason, today there is a possibility of accidental detection of an IUD that cannot be shown on a two-dimensional (2D) ultrasound. The use of 3D three-dimensional ultrasound helps us to accurately display the uterine cavity and its external contours. This clinical case highlights the importance of three-dimensional ultrasound in the diagnosis of a retained intrauterine device

Case report

A 66-year-old patient presented to the clinic with a suspicion of endometrial polyp. The patient has given birth vaginally twice, had her last period in 2005, and denies postmenopausal bleeding. A more detailed anamnesis revealed that she had an IUD inserted since 1981. She did not report vaginal bleeding, discharge or pain. She had not had any gynecological procedures, abdominal or pelvic surgeries. A 2D ultrasound examination showed hyperechoic intermittent echoes with posterior shadowing in the uterine cavity, without detectable flow in Color Doppler. The endometrial-myometrial border was not completely clearly defined, it was partly irregular and intermittent, sonomorphological characteristics of adenomyosis. A three-dimensional transvaginal ultrasound was then performed, which enabled more precise visualization. The 3D multiplanar-coronal view showed echoes for an IUD with an unusual coil shape, which would correspond to the Beospir IUD in terms of characteristics, and ruled out adenomyomic changes and the presence of endometrial polyps. The patient was referred for surgery, hysteroscopic extraction of the IUD. Due to the stenosis of the external os of the cervix, the procedure could not be performed, and it was decided to continue ultrasound monitoring of the asymptomatic patient.

Discussion

Intrauterine devices retained for longer than the intended time are associated with numerous complications such as infection, bleeding, chronic pelvic pain and pyometra. However, there are cases without symptoms even though the IUD has been retained in the uterus for years. In our case, the patient had no symptoms, and removal of the IUD is not necessary. An IUD can be retained or forgotten for too long in the uterus for several reasons, and one of the main reasons is the lack of complete communication during the insertion itself, poorly maintained documentation and inadequate gynecological monitoring of patients with an IUD inserted. For this reason, some patients forget that they have an IUD. Transvaginal ultrasound should be able to show all the structures of the uterus, including the presence of the IUD in the uterus. However, sometimes ultrasound imaging of the IUD is not always easy due to the different types and shapes of IUDs. This is the importance of three-dimensional ultrasound, which can show the uterus in a multiplanar-coronal plane, thus showing all the planes and structures within the uterine cavity, and sparing the patient further exhausting diagnostic procedures.

Conclusion

This case report confirms the diagnostic value of three-dimensional ultrasound. Three-dimensional ultrasound improves diagnostic accuracy, allowing for more detailed and precise visualization of uterine structures. Studies show that 3D ultrasound is more sensitive and specific than two-dimensional ultrasound in defining and mapping uterine lesions. Its sensitivity and specificity are close to 100% for diagnosing congenital uterine anomalies. The use of 3D ultrasound in routine gynecological examinations can be beneficial for clinicians because it provides fast and accurate data in real time and is more cost-effective than other diagnostic tests such as magnetic resonance imaging (MRI).

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Two follicles, two oocytes, twin pregnancy: a successful pregnancy after IVF journey in a patient with diminished ovarian reserve and endometriosis

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Abstract

We present a case of a successful twin pregnancy achieved via in vitro fertilization (IVF) in a patient diagnosed with diminished ovarian reserve (DOR) and endometriosis. The patient underwent controlled ovarian stimulation using a modified long GnRH agonist protocol with recombinant LH supplementation, resulting in the retrieval of only two oocytes from two follicles. Both oocytes fertilized successfully, and the resulting high-quality cleavage-stage embryos were transferred fresh on day 4 of culture. The decision to transfer both embryos was made after thorough counselling on success rates and risks, including the possibility of multiple pregnancy, and in accordance with the patient's request. This case highlights that, even in patients with markedly reduced ovarian reserve and endometriosis, the combination of tailored stimulation protocols, optimal endometrial preparation, and patient-centered decision-making can lead to a successful pregnancy outcome.

Introduction

Diminished ovarian reserve and endometriosis are two significant and often interrelated causes of infertility. DOR, characterized by a reduced quantity and quality of oocytes, limits the success of assisted reproductive technologies (ART). Endometriosis, particularly in advanced stages, can impair both ovarian function and endometrial receptivity. Patients with both conditions typically have lower chances of conception through IVF, with reduced oocyte yield and fertilization potential. However, controlled ovarian stimulation protocols, including the long GnRH agonist protocol, may improve outcomes by suppressing endometriotic activity and optimizing follicular development. This case presents a remarkable IVF success story in a patient with poor prognostic factors, resulting in the birth of healthy twins from just two retrieved oocytes.

Case presentation

The patient was a 38-year-old woman who had been struggling with primary infertility for two years. She had a medical history significant for stage II endometriosis, which was diagnosed by sonographic assessment, presenting with multiple endometriomas on both her ovaries. Her menstrual cycles were regular, occurring every 28 to 30 days. On evaluation, her ovarian reserve was found to be diminished. Anti-Müllerian hormone (AMH) levels were low at 0.7 pmol/L, and the antral follicle count (AFC) was four, with two follicles observed in the right ovary and two in the left ovary. On day 3 of her menstrual cycle, her FSH level was 26 mIU/mL, and her LH level was 11 mIU/mL. Thyroid function tests revealed a TSH level of 3.57 mIU/L, which was elevated for a patient attempting conception. Semen analysis of her partner was within normal limits. Given her diagnosis, the decision was made to proceed with in vitro fertilization (IVF) using a long GnRH agonist protocol to optimize ovarian response and suppress endometriosis activity.

IVF Treatment

Three months before the successful treatment cycle, the patient underwent her first IVF attempt using a long GnRH agonist protocol with 300 IU rFSH. Despite adequate duration of stimulation and monitoring, the ovarian response was poor, and only one follicle developed to a dominant

size, resulting in one oocyte and no fertilisation after retrieval. The patient was counseled about her diminished ovarian reserve and the potential benefits of adjusting the stimulation protocol. She declined oocyte donation and opted for a second IVF attempt using a modified approach. In the subsequent cycle, conducted three months later, a long GnRH agonist protocol was again used for pituitary downregulation. Triptorelin acetate (0.1 mg subcutaneously) was initiated in the mid-luteal phase. Once downregulation was confirmed, ovarian stimulation commenced with 300 IU of recombinant FSH and recombinant LH, administered daily. The addition of recombinant LH was intended to support follicular development, particularly in the context of poor ovarian reserve, where LH supplementation may enhance oocyte maturation and estrogen production. Final oocyte maturation was triggered with 250 mcg of choriogonadotropin alfa, and oocyte retrieval was performed 36 hours later. During the retrieval, two oocytes were successfully obtained from two follicles on the right ovary, while no follicles were seen on the left ovary. Both oocytes were fertilized using intracytoplasmic sperm injection (ICSI), and the resulting embryos were cultured in vitro. By day 4 of culture, both embryos had reached the late cleavage stage. Prior to embryo transfer, elective single embryo transfer (eSET) was recommended. The patient and her partner were thoroughly counseled regarding the potential outcomes, success rates, and risks associated with transferring two embryos (double embryo transfer, DET) in a single cycle, including the heightened chance of multiple pregnancy and associated obstetric implications. The couple expressed a strong preference for transferring both embryos in the same cycle to maximize the likelihood of pregnancy, and this decision was respected following the provision of written informed consent. A fresh transfer of both cleavage-stage embryos was then performed under ultrasound guidance. The endometrial thickness measured 11 mm with a favorable trilaminar pattern. Luteal phase support was initiated on the day of oocyte retrieval using vaginal micronized progesterone at a dose of 400 mg twice daily.

Outcome and Follow-up

Serum beta-hCG testing performed 14 days post-transfer confirmed a positive pregnancy. A transvaginal ultrasound at six weeks' gestation revealed a viable dichorionic diamniotic twin pregnancy, with two intrauterine gestational sacs and fetal heart activity visible in both embryos. The pregnancy progressed without major complications. The patient received regular antenatal care and fetal growth assessments. At 37 weeks of gestation, she delivered two healthy infants via elective cesarean section. Both neonates had appropriate birth weights and Apgar scores, and no neonatal intensive care was required.

Discussion

This case demonstrates that careful protocol selection, individualized treatment planning, and comprehensive patient counselling can result in favorable outcomes, even when only two oocytes are retrieved. In ovarian stimulation procedures for patients with poor ovarian response, the most commonly used protocols include GnRH agonists and GnRH antagonists. Meta-analyses conducted in 2014 and 2017 found no statistically significant differences in live birth rates between long GnRH agonist and antagonist protocols. While some studies, such as that by Sunkara, have suggested that long agonist protocols may yield a higher number of mature oocytes, these trends have not reached statistical significance. Despite the general parity between the two protocols, the long GnRH agonist protocol may offer specific benefits for certain patient subgroups, particularly those with endometriosis and DOR.

A recent systematic review by Kuan reported that most studies show comparable clinical pregnancy and live birth rates between agonist and antagonist protocols, with similar fertilization rates. However, for women with advanced endometriosis, the long agonist approach may improve outcomes. Kolanska et al. observed a significantly higher clinical pregnancy rate per started cycle in women with various forms of endometriosis undergoing fresh embryo transfers with the agonist protocol.

In this case, the initial IVF attempt resulted in a poor ovarian response. In contrast, the second cycle employed a modified long GnRH agonist protocol with the addition of recombinant LH, which may have supported better follicular recruitment and oocyte maturation, particularly in the context of DOR. Furthermore, the suppression of endometriotic activity via prolonged GnRH agonist exposure could have improved endometrial receptivity, which may also have contributed to successful implantation following fresh embryo transfer.

Although elective single embryo transfer (eSET) is generally recommended to minimize the risk of multiple gestation and associated complications, in this case, a double embryo transfer (DET) was performed due to specific circumstances. The patient had only two cleavage-stage embryos available for transfer and, given her advanced maternal age (38 years) and history of diminished ovarian reserve with poor ovarian response, the clinical team faced a difficult balance between maximizing pregnancy chances and minimizing risks.

An important aspect of the management in this case was the thorough pre-transfer counselling, during which the patient and her partner were informed of the realistic chances of success, potential complications, and specific risks of multiple pregnancy associated with transferring two embryos. The couple expressed a strong preference for transferring both embryos in the same cycle to maximize the likelihood of pregnancy. Ultimately, the combination of a tailored stimulation protocol, rLH supplementation, optimal endometrial preparation, and a patient-centered counselling process contributed to the successful twin pregnancy.

Conclusion

Patients with diminished ovarian reserve represent one of the most complex challenges in reproductive medicine. åDespite significant advancements in medical science and assisted reproductive technologies, it remains essential to evaluate the complete clinical picture and detailed medical history. Based on this comprehensive assessment, the most appropriate and individualized treatment strategy should be determined for each couple, with the aim of optimizing reproductive outcomes.

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Endocrinological aspects of reproductive-age patients with early endometrial cancer.

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Introduction

Endometrial carcinoma is one of the most common malignant tumors in women and the sixth most common tumor in the female population. It primarily affects older women, with the highest incidence between the ages of 55 and 64. However, recent studies indicate an increasing number of reproductive-aged women diagnosed with endometrial carcinoma, occurring in about 5% of women younger than 40 years. It is associated with increased estrogen production without the opposing effect of progesterone. Risk factors include early menarche, late menopause, anovulation, nulliparity, obesity, type II diabetes, and PCOS. PCOS is a complex endocrinological disorder of reproductive-aged women, with a prevalence of 4–21%, recognized as a risk factor for endometrial cancer. Hyperandrogenic anovulation is thought to cause the conversion of androgens into estrogens, leading to increased mitotic activity and endometrial cell proliferation.

The aim of this study was to examine the endocrinological aspects of patients diagnosed with atypical endometrial hyperplasia or endometrial carcinoma, with particular emphasis on polycystic ovary syndrome (PCOS).

Materials and Methods

This was a cross-sectional clinical study conducted between 2012 and 2024 at the UKCS Clinic for Gynecology and Obstetrics. The study included 62 patients aged 45 years and older. Insulin resistance was assessed using HOMA-IR (>2.6). PCOS was diagnosed according to the ESHRE/ASRM criteria. PCOS phenotypes were classified as A, B, C, and D. Data analysis was performed using SPSS 13.0 and descriptive statistical methods.

Results

Histopathological analysis of endometrial samples revealed atypical hyperplasia in 18 patients (29%), EIN in 18 patients (29%), and endometrial carcinoma in 26 patients (42%). Among conservatively treated patients, 22 (42.3%) had PCOS. Control histopathological findings after initial therapy were normal in 51.6% of patients. Disease recurrence was diagnosed in 23 patients (44.23%), occurring with similar frequency (around 30%) during the first and second years of follow-up as well as after this period. Recurrence was most common in women with phenotype A (60%), while it was not observed in women with phenotype D.

Discussion

PCOS is a heterogeneous disorder with various clinical phenotypes, characterized by hyperandrogenism, insulin resistance, and hyperinsulinemia, which can ultimately lead to malignant transformation. Obesity accounts for 34% of the global incidence of endometrial cancer. In developed Western countries, obesity contributes to over 40% of cases, compared to less than 20% in Asia. The combination of hyperestrogenism, inflammation, and obesity-induced insulin resistance creates a metabolic environment that drives tumorigenesis. Establishing guidelines for regular testing of insulin resistance in obese patients during annual gynecological examinations could be beneficial in preventing endometrial cancer.

Conclusion

The results of our study suggest that by assessing the endocrinological status of patients with endometrial carcinoma, along with appropriate dietary and lifestyle interventions, conservative therapy, and psychosocial support, favorable therapeutic outcomes can be achieved while preserving fertility in reproductive-aged patients.

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Ovarian stromal hyperthecosis: an endocrine cause of hyperandrogenism and metabolic dysfunction in postmenopause

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Introduction

Hyperandrogenism in postmenopause is rare and requires differential diagnostic evaluation due to potential neoplastic (adrenal or ovarian) or functional causes, among which is ovarian stromal hyperthecosis. This benign condition is characterized by diffuse proliferation and luteinization of theca cells, leading to increased androgen production. Clinical manifestations include virilization (hirsutism, androgenic alopecia) and metabolic disturbances such as insulin resistance and diabetes mellitus. Androgens reduce peripheral insulin sensitivity, which may worsen glycemic control, particularly in women with preexisting diabetes. In the diagnostic process, the GnRH agonist test may help distinguish a functional, LH-dependent source from an autonomous source of androgens, such as an ovarian or adrenal tumor.

Aim

To present a rare case of ovarian stromal hyperthecosis as a cause of postmenopausal hyperandrogenism, to emphasize the importance of the GnRH agonist test in differentiating ovarian from adrenal sources of androgen excess, and to illustrate the impact of hyperandrogenism on glycemic status.

Case

We present a 67-year-old postmenopausal woman with clinically pronounced hyperandrogenism (FG score 15). Her medical history included arterial hypertension, panic disorder, and type 2 diabetes mellitus (HbA1c 5.3%) treated with metformin. Hormonal evaluation revealed elevated total testosterone (6.4 nmol/L), 17-OHP, estradiol, and progesterone, with normal DHEA-S. Ultrasound showed enlarged ovaries without folliculogenesis. The GnRH agonist test (goserelin 3.6 mg s.c.) resulted in gonadotropin suppression and a drop in testosterone to 0.6 nmol/L, confirming an ovarian, LH-dependent source of androgens. Laparoscopic bilateral adnexectomy was performed. Histological analysis demonstrated diffuse proliferation of luteinized theca cells, confirming the diagnosis of stromal hyperthecosis. Postoperatively, complete normalization of androgen levels and significant improvement in glycemic control were achieved, allowing discontinuation of metformin therapy.

Conclusion

Ovarian stromal hyperthecosis is a rare but clinically important cause of postmenopausal hyperandrogenism. The GnRH agonist test is crucial in differentiating the etiology of hyperandrogenism. Timely recognition enables targeted treatment and reversible normalization of both hormonal and metabolic status. It is important to recognize the role of hyperandrogenism as a contributor to metabolic dysfunction and cardiovascular risk.

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Incidence and delivery methods of twin pregnancies over a two-year period at the University Hospital of Split

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Objective

The primary aim of this study was to analyze the incidence and modes of delivery of twin pregnancies at the University Hospital Centre Split during 2023 and 2024, with emphasis on differences between spontaneously conceived and medically assisted reproduction (MAR) twin pregnancies. Secondary aims included comparing the distribution of placentation types (dichorionic and monochorionic), gestational duration, and the influence of prior delivery and other clinical factors on the choice of delivery method.

Materials and methods

This research was conducted as a retrospective cross-sectional study on a sample of 160 women who delivered twins at the University Hospital Centre Split during 2023 and 2024. Data were collected from delivery room records and the hospital information system, and included demographic and clinical variables: maternal age, mode of conception (spontaneous or MAR), parity, gestational age, type of twin pregnancy, mode of delivery (vaginal, emergency or elective cesarean section, combined delivery), and neonatal outcomes.

Results

Twin pregnancies accounted for 2% of all deliveries (160 out of 7986), with 109 conceived spontaneously (68.1%) and 51 through MAR (31.9%). The overall cesarean section rate was 88.1% (44% elective and 43% emergency), while vaginal deliveries accounted for 12% and combined deliveries for 1%. Vaginal delivery was more common in spontaneously conceived pregnancies (14.7%), whereas the majority of MAR twin pregnancies (94.1%) were delivered by cesarean section. Differences in cesarean section rates between groups were not statistically significant (P=0.109). Women in the MAR group were significantly older (median 35 compared to 30 years, P<0.001). There were more primiparous women in the MAR group (62.7% compared to 47.7%, P=0.076). Dichorionic pregnancies were more frequent in the MAR group (86.3% compared to 78.9%), but without statistical significance. Chorionicity showed no significant association with the mode of delivery. The preterm birth rate was 50.6%, with no difference between groups. Perinatal outcomes showed a high proportion of live births (96.3%) without differences between spontaneous and MAR pregnancies. Previous cesarean section was the strongest predictor of operative delivery - none of the women with a prior cesarean delivered vaginally.

Conclusion

The majority of twin pregnancies at the University Hospital Centre Split were delivered by cesarean section, significantly exceeding international guideline recommendations. The mode of conception (spontaneous or MAR) had no statistically significant impact on the choice of delivery method, although women in the MAR group were significantly older and more often primiparous. Chorionicity

was not significantly associated with the mode of delivery. Preterm birth rate did not differ depending on the mode of conception. Favorable perinatal outcomes and the high rate of live births point to high-quality perinatal care, with a need for critical re-evaluation of current practices and encouragement of increased rates of vaginal deliveries in twin pregnancies.

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Ovarian collision tumor in a postmenopausal patient; mucinous cystadenoma and monodermal teratoma in a single mass

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Introduction

Mucinous tumors of the ovary vary from benign cystadenomas, borderline tumors to malignant adenocarcinomas (1). Mucinous cystadenomas are characterised by mucin- producing epithelial cells. They are mostly benign (80%) and frequently asymptomatic at early stages (2). Monodermal teratomas are either positive for mature thyroid tissue (struma ovarii) or for carcinoid tissue (carcinoid neoplasms). Ovarian collision tumors are rare neoplasms which are mixtures of different combinations of epithelial tumors, germ cell tumors, and sex-cord-stromal tumors (3). We shall present a curious case of mucinous cystadenoma and monodermal teratoma in a single mass in a postmenopausal patient.

Case report

In February 2025 a 54 year old postmenopausal female patient was admitted to our Department because of surgical treatment of a right ovarian tumor. The tumor was initially diagnosed on a routine annual check up at a primary gynaecologist in November 2024, and the patient was then referred to our outpatient clinic for further diagnostic and curative procedure. In her personal history, the patient has had two cesarean sections, has always had normal PAP smears, her last menstrual period was two years ago and she has not bled since. She has chronic arterial hypertension regulated with antihypertensive therapy and Hashimoto thyroiditis, also regulated therapeutically. The patient did not have any symptoms regarding the ovarian tumor and her family history was negative for gynaecological neoplasms. In our outpatient clinic, the ultrasound finding showed right ovarian tumor 4x4 centimeters with thick septi and positive color Doppler score.

Endometrial lining was thin, left ovary and the rest of the anatomy in order. The patient was advised to check tumor markers; CEA, CA-125, CA 19-9, CA 15-3 and ROMA index, which were all normal. However, considering the ultrasound finding, we decided to treat the patient surgically. In February 2025 the patient underwent billateral laparoscopic adnexectomy. During the procedure, cytologic aspiration of the right ovarian tumor was performed, and aftwerards both adnexa were removed separately in endoscopic bags without any leftovers or spillage. Cytologic finding came positive for mucinous cystadenoma while pathohistological findings confirmed monodermal teratoma and mucinous cystadenoma in a single tumor mass. The left adnexal pathohistological findings showed a small simple ovarian cyst.

Conclusion

Ultrasound imaging of collision tumors can be challenging because the smaller tumor might be ''nested'' inside a larger one or even placed ''back to back'', i.e. on the wall of a larger tumor (3). These tumors are best viewed on CT or MRI since each type of tumor presents specific features in accordance with their histologic type (3). The radiologic recognition of collision tumors is essential to ensure comprehensive biopsy and therefore optimal treatment. In our patient, the monodermal teratoma was positive for mature tyroid tissue (struma ovarii) and postoperative thyroid hormone check up was normal.

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Serum progesterone variations in ovarian stimulation with highly purified HMG versus recombinant FSH: results from real-life practice

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Aim

We aimed to compare serum progesterone levels in follicular phase on triggering day and IVF outcomes in patients undergoing either highly purified Human Menopausal Gonadotropin (hp-HMG) or recombinant Follicle-Stimulating Hormone (r-FSH) stimulation protocol.

Background

High levels of progesterone in the follicular phase were found to be associated with less successful IVF outcomes in terms of live birth rates. Compared to cycles stimulated with r-FSH, hp-HMG stimulated cycles were found to have lower serum progesterone in the follicular phase on the day of triggering and higher levels of serum estradiol. The difference in serum progesterone levels between these two ovarian stimulation protocols is thought to be either due to different steroidogenesis pathways or due to stronger ovarian response in r- FSH stimulated cycles.

Patients and methods

We performed a retrospective observational single-center study on 115 women who underwent ovarian stimulation (OS) protocols with GnRH antagonists and either hp-HMG (n=27) or r-FSH (n=72). The doses of the drugs used were individualised according to the ovarian reserve test. Women included in the study were treated from January to December 2019 in our University clinic. Participants were between 22 and 42 years of age and were undergoing OS for infertility reasons. In accordance with modern practice, OS was initiated on the second day of menstrual cycle and continued until the minimal follicular size was 17 mm. The aspiration was performed 24 to 48 hours after the trigger (choriogonadotropin alpha) was applied. Serum progesterone and estrogen levels were determined on the first day of ovarian stimulation and on the day of triggering.

Results

Patients that underwent OS with r-FSH had significantly lower progesterone serum levels than those that underwent OS with hp-HMG (2,39 1,91ng/mL vs 3,08 6,59ng/mL respectively, P=0,007). No significant difference in IVF outcomes, i.e. live births between the two OS protocols was detected (29,2% vs 33,3% respectively, P=0,689). In a multivariable regression analysis, only age of the patients was shown to be associated with a successful IVF outcome (age <40y, Exp(B) for live birth = 4,01, 95%CI 1,02-15,69, P=0,046). No relevant bias (with an impact on the aforementioned results) was identified.

Conclusion

The findings of our study suggest that higher levels of serum progesterone can be achieved when OS is performed using hp-HMG. Our findings also suggest that serum progesterone levels are not associated with the success of IVF. Baring in mind the results of previous trials, further research is warranted.

Acknowledgements

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Detection of Pallister-Killian Syndrome via Genome-wide NIPT – two case reports

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Objective

Pallister-Killian syndrome (PKS) is a rare sporadic genetic disorder characterized by tissue-specific mosaic distribution of an extra isochromosome 12p. Its prevalence is estimated at 1 in 20,000. It is not inherited and occurs spontaneously. Typically, children with this diagnosis present with severe hypotonia, intellectual disability, developmental delay, distinct facial features, pigmentary skin differences, seizures, and other congenital anomalies. Due to the low recurrence risk, prenatal diagnosis of this syndrome is essential for genetic counseling. The aim of this paper is to present the first two cases of Pallister-Killian syndrome detected through genome-wide NIPT in Bosnia and Herzegovina.

Methods

The first case involved a 30-year-old woman with two prior miscarriages, referred to our genetic counseling team at 13 weeks of gestation due to her medical history. A year earlier, she had experienced a miscarriage of a twin pregnancy caused by twin-to-twin transfusion syndrome. In the current pregnancy, ultrasound examination had shown no abnormal markers.

The second case involved a 31-year-old woman in her second pregnancy, referred for prenatal testing due to increased nuchal translucency at 10 weeks (NT 2.3 mm). Based on medical history and ultrasound findings, our geneticist recommended genome-wide NIPT for both cases. This test analyzes all chromosomes for structural and numerical abnormalities in the fetal genome.

Results

In the first case, the NIPT result (14th week) was positive for a partial duplication (CNV) on chromosome 12: dup(12)(p13.33p11.1). This region corresponded to the entire short arm (p-arm) of chromosome 12. The fetal fraction was 11%. Genetic counseling was subsequently provided to the gynecologist and patient. To confirm the diagnosis, invasive prenatal testing (amniocentesis) was performed despite the miscarriage risk. The analysis confirmed the diagnosis in cultured amniotic cells. Standard GTG banding revealed a mosaic female karyotype with two cell lines: one with a female karyotype carrying an additional chromosome i(12p) in 8 of 16 metaphase cells, and the other with a normal female karyotype.

In the second case, the NIPT result (11th week of gestation) was positive for a partial duplication (CNV) on chromosome 12: dup(12)(p13.33q12). The duplication size was 37 Mb. The fetal fraction was 9%. The patient experienced bleeding after a follow-up ultrasound and increased nuchal translucency, which ultimately resulted in miscarriage.

Conclusion

Prenatal diagnosis of Pallister-Killian syndrome is challenging by cytogenetic analysis and even by ultrasound. We demonstrated the capability of next-generation genome-wide NIPT (non-invasive prenatal testing) to provide early suspicion and facilitate subsequent genetic confirmation of PKS. As genome-wide NIPT becomes more accessible, incidental detection of partial aneuploidies is expected to increase.

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Evaluation of two Single-step embryo culture media: early developmental differences without clinical impact

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Objective

To compare the performance of two commercially available single-step embryo culture media—SAGE 1-Step™ and global® total®—with respect to embryo quality and major clinical outcomes such as fertilization, implantation, blastulation and clinical pregnancy rates in IVF/ICSI cycles.

Materials and Methods

This retrospective randomized sibling oocyte split study included all mature (MII) oocytes retrieved from women undergoing IVF/ICSI treatment at the Clinical Hospital Center “Sestre milosrdnice” (Zagreb, Croatia) between January 2021 and December 2022. A total of 915 oocytes from 110 patients were randomly allocated post-fertilization to either system A (SAGE 1-Step™, Origio-CooperSurgical, Denmark) or system B (global® total®, Origio-CooperSurgical, Denmark). Embryo development was assessed until day 3 (D3) and day 5 (D5). Comparative analyses of embryo quality and clinical outcomes were conducted using z, chi-square and Fisher's exact tests (Stata/SE 16.1).

Results

Embryos cultured in global® total® media showed significantly higher proportions of good-quality embryos on day 2 (71.37% vs. 80.88%; p=0.0103) and day 3 (66.67% vs. 75.74%; p=0.0214) compared with SAGE 1-Step™ media. Clinical pregnancy rate (21.54% vs. 22.03%; p=0.9463) and implantation rates (20.00% vs. 24.14%; p=0.618) were higher in the global® total® group, although the differences were not statistically significant. Blastocyst formation rate (52,46% vs. 53,50%, p = 0,890) and fertilization rate (34,41% vs. 34,27%; p=0.955) did not significantly differ between the two media. The difference in embryo utilization between SAGE 1-Step™ and global® total® (63.92% vs. 58.09%; p = 0.20) also showed no statistical significance.

Conclusion

The findings indicate that the choice of culture medium may influence early embryo development, particularly in terms of cleavage-stage quality, while blastocyst development and clinical outcomes remained comparable. Given the retrospective design and limited sample size, these results warrant confirmation in larger prospective studies to further evaluate the clinical relevance of single-step media selection in human embryo culture.

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Polycystic Ovary Syndrome and Obesity

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age and is often associated with metabolic syndrome. Obesity, particularly when it begins in adolescence, further disrupts hormonal balance and contributes to the development of insulin resistance and clinical signs of hyperandrogenism.

Case Report

A 19-year-old female patient, with a history of excess body weight since childhood and under pediatric follow-up, was referred for endocrinology consultation due to obesity. Secondary causes of obesity had previously been excluded. Earlier attempts at weight reduction with nutritional counseling and increased physical activity led to fluctuations in body weight but no long-term results. Menarche occurred at the age of 12, and since age 14 she has had irregular bleeding every 4–5 months. On clinical examination, in addition to central obesity and BMI of 44 kg/m^2, acanthosis was observed in the axillae and on the neck, indicative of insulin resistance, along with pronounced pale striae on the abdomen and increased hair growth on the face and upper arms (Ferriman-Gallwey score 4). Laboratory findings confirmed insulin resistance (HOMA-IR 4.6) and hyperandrogenemia (total testosterone 2.2 nmol/L). Ultrasound showed normal ovarian morphology. According to the Rotterdam criteria, the patient meets the requirements for the diagnosis of PCOS (oligomenorrhea and hyperandrogenemia), phenotype C. Metformin and dydrogesterone had previously been introduced into therapy, resulting in scant bleeding.

Treatment plan

The main therapeutic goal for this patient is weight reduction. Previous studies have shown that a 5% weight loss can lead to a 35% decrease in circulating testosterone levels and a 40% improvement in insulin sensitivity. Treatment options for obesity include lifestyle modification (which has not yielded results so far), pharmacotherapy, and bariatric surgery. The patient was recommended obesity pharmacotherapy with a GLP-1 receptor agonist, semaglutide, which has been shown to result not only in weight loss but also in cycle normalization. Since semaglutide is not approved for use during pregnancy, the patient must be advised on the necessity of contraception; hormonal contraception may be used simultaneously, which also has therapeutic benefits in PCOS. Alongside pharmacotherapy, it is essential to continue lifestyle modification (dietary adjustments and increased physical activity), and if satisfactory results are not achieved, bariatric surgery remains an option.

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Late onset OHVIRA syndrome treated with hymen-sparing vaginoscopic incision of vaginal septum

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Introduction

OHVIRA (Obstructed Hemivagina with Ipsilateral Renal Anomaly) syndrome (Herlyn-Werner-Wunderlich syndrome) is a rare congenital anomaly of the female urogenital tract. It results from abnormal development of the Müllerian ducts and Wolffian duct system. Typically presents at menarche or shortly after with cyclical abdominal pain, pelvic mass, dysmenorrhea and sometimes urinary symptoms.

Case presentation

We present an 18 years old female with urinary retention, with prior uneventful gynecological history and regular menstrual bleeding. Initial work up discovered hyperechogenic pelvic tumor measuring 10x11cm (presumably ovarian tumor), elevated tumor markers (CA-125 182.8 U/mL; HE4 62 pmol/L; CA 19-9 149,6 U/mL) and absent right kidney. On MRI uterine anomaly was found, the patient has 2 corpus of uterus, 2 cervixes, longitudinal obstructing vaginal septum (ESHRE/ESGE classification U3b, C2, V2) and kidney agenesis. The suspected tumor was hematocolpos and it caused urethral compression. Hymen-sparing vaginoscopy was done with incision of septum and drainage of hematocolpos. The patient was discharged the next day from hospital without urinary retention and gynecological difficulties. In the 1 month postoperative follow-up patient was without any symptoms. The transabdominal ultrasound did not detect the previously described hematocolpos.

Discussion

The presentation of late onset symptoms and elevated tumor markers was a confusing factor in diagnostic workup. However, MRI and following vaginoscopy enabled us to exclude pelvic tumors and to visualise and treat this rare condition. Hymen-sparing procedures are surgical techniques that allow the diagnosis and treatment of vaginal, cervical and also uterine cavity issues in women who wish to maintain the integrity of their hymen.

In conclusion

Vaginoscopic incision of the vaginal septum is a safe, minimally invasive, and effective approach for treating OHVIRA syndrome in adolescents with hematocolpos. This technique should minimize disruption to the vaginal wall and postoperative pain while providing appropriate visualization throughout the procedure.

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Fertility-sparing treatment in a premenopausal patient with endometrial cancer: a case report

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Introduction

Endometrial cancer is the most common gynaecologic malignancy, occurring in approximately 7% of premenopausal women. While hysterectomy remains the gold standard of treatment, fertility- sparing management represents a reasonable option for patients diagnosed with atypical endometrial hyperplasia or FIGO stage IA non-invasive endometrial carcinoma.

Case Report

We present the case of a 41-year old woman with a known history of Hashimoto thyroiditis and an otherwise unremarkable medical background. Her family history is notable lung carcinoma and sarcoma. In 2021, the patient underwent laparoscopic removal of a left ovarian teratoma. During the same procedure, hysteroscopic resection of an incomplete uterine septum was performed. One year later, a transvaginal ultrasound identified a 3 centimeter endometrial polyp. Hysteroscopy was subsequently performed, and pathohystological analysis revealed the presence of endometrial adenocarcinoma. The patient was nulliparous but expressed a strong desire for future fertility.

Therefore, a fertility-sparing approach was chosen. The patient was treated with megestrol acetate 160 mg daily for three months, followed by the placement of a levonorgestrel-releasing intrauterine device (IUD). In accordance with her reproductive goals, controlled ovarian stimulation was performed one year after the initiation of conservative treatment, resulting in the cryopreservation of six embryos, with an IUD remaining in situ throughout the process. A second-look hysteroscopy and MRI control were performed to confirm complete response prior to embryo transfer. Following the removal of the IUD, a frozen embryo transfer was performed in a natural cycle and pregnancy was successfully achieved. The course of the pregnancy was uneventful until the third trimester, when the patient was re-admitted at 31 weeks of gestation due to vaginal bleeding, which was followed by preterm premature rupture of membranes. She was hospitalised and treated conservatively for two weeks, during which she received antibiotic therapy, dexamethasone for fetal lung maturation, and tocolysis. At 34 weeks of gestation, due to the progression of clinical symptoms, a cesarean section was performed. A live female preterm neonate was delivered with reassuring Apgar scores 9/10.

Discussion

Premenopausal patients diagnosed with endometrial carcinoma who have not yet completed childbearing represent a significant therapeutic challenge. Fertility-sparing management offers a valuable treatment option in selected cases and is primarily based on hysteroscopic evaluation combined with the use of a levonorgestrel-releasing IUD. Due to low-certainty evidence, the optimal type, dose, and route of progestin therapy for fertility-sparing management in endometrial cancer or atypical endometrial hyperplasia remain unclear. The levonorgestrel-releasing IUD appears similarly effective to oral progestins, with fewer side effects, potentially improving quality of life and treatment adherence (2).

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Against the Odds: A Case of Successful IVF with Own Eggs in the Mid-40s Despite Low Ovarian Reserve and Repeated Failures

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Introduction

The decision to discontinue attempts at conception with autologous oocytes is challenging for both patients and clinicians and is influenced by multiple factors, including age, ovarian reserve, treatment history, comorbidities, financial resources, and psychological well-being. Although some guidelines suggest three to four IVF cycles, there is no universally accepted upper limit. Cumulative live birth rates continue to increase with successive attempts, and many couples require up to six cycles to achieve a successful outcome. Patient age, repeated implantation failure, and diminished ovarian reserve are the strongest predictors of poor prognosis, yet individualized counseling and shared decision-making remain essential, as favorable outcomes are occasionally achieved despite unfavorable baseline parameters.

Case

A 42-year-old woman and her 43-year-old partner presented to our Center for Reproductive Medicine after seven years of primary infertility. They had previously undergone five IVF cycles, one resulting in a clinical pregnancy that ended in miscarriage at eight weeks' gestation. Two years earlier, the patient had been diagnosed with hyperprolactinemia and treated with bromocriptine, with subsequent normalization of prolactin levels. Family history was notable only for type 2 diabetes mellitus and hypertension in her mother. One year before presentation, she underwent hysteroscopic resection of an endometrial polyp.

During the following two years, seven additional ovarian stimulation cycles were performed using various protocols. The number of retrieved oocytes ranged from 0 to 8. Except for one cycle, all attempts involved fresh single-embryo transfers (SET) at cleavage or blastocyst stage. In two cycles, surplus blastocysts were cryopreserved and later transferred, resulting in a total of nine SETs, all unsuccessful.

At nearly 45 years of age, the patient elected to pursue another attempt. Baseline ovarian reserve assessment showed AMH 1.0 pmol/L, FSH 12.2 IU/L, and AFC 2. Mild ovarian stimulation was initiated with rFSH 150 IU daily from day 3 to day 8, followed by HCG (6500 IU SC). Two follicles were aspirated, yielding a single mature oocyte, which was fertilized by ICSI. On day 3 post-aspiration, at the morula stage, the embryo was transferred.

A positive β-hCG was documented two weeks later, with clinical confirmation of pregnancy one week thereafter. The subsequent course was uneventful. Non-invasive prenatal screening demonstrated low risk for common aneuploidies, and the pregnancy progressed into the third trimester without complications.

Conclusion

This case highlights that oocyte competence, rather than ovarian reserve parameters alone, is the decisive factor determining IVF success. Even in women of advanced reproductive age with diminished reserve and multiple previous failures, favorable outcomes are achievable. The report

underscores the importance of individualized stimulation strategies, careful embryo selection, and patient persistence in achieving a live birth.

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Extracellular Vesicles in Spent Embryo Culture Medium: Characterization and Considerations for Reliable Biomarker Discovery

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Extracellular vesicles (EVs), nanoscale lipid bilayer-bound particles mediating intercellular communication, have emerged as promising non-invasive biomarkers in assisted reproductive technologies. Embryos release EVs into culture medium, and their molecular cargos may reflect developmental competence and embryo–maternal signaling, supporting their potential application in embryo assessment and selection as alternatives or complements to invasive methods. Despite this, reported controls often insufficiently distinguish embryo-derived EVs from artefactual or non-vesicular extracellular particles arising from culture media, handling, or technical procedures. This omission risks confounding the interpretation and reproducibility of results across studies.

To address this, we aimed to discriminate bona fide embryo-derived EVs from artefactual structures. Accordingly, we established a multi-modal analytical framework that integrates transmission electron microscopy (TEM) and nano-flow cytometry applied to both spent embryo culture media and rigorously defined negative controls.

Preliminary TEM imaging confirmed the presence of vesicle-like structures in embryo-conditioned media while simultaneously revealing distinct morphological features in controls. These findings highlight the necessity of comprehensive baseline characterization in line with current MISEV2023 recommendations, ensuring that vesicular signatures can be confidently attributed to embryonic origin.

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