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Editor in Chief

Lucia De Santis

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Research Innovation Views Embryology Reproduction

Editor in Chief

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EDITORIAL

The Italian Society of Fertility and Sterility, SIFES, was founded in 1965 and has always had the objective of promoting research and studies on reproductive medicine and biology to improve the therapeutic possibilities for infertile couples. Society also deals with examining all related ethical and social issues, also promoting initiatives to achieve the adoption of certain legislative rules on the matter.

This year too, the Society is celebrating its annual congress structured in different sessions and symposia.

The Opening Symposium will focus on a highly relevant topic: how communication in Reproductive Medicine has changed, how it can be improved, and what it means for patients' understanding and well-being.

Four additional invited Symposia are dedicated to topics currently being explored by some of the Society's Special Interest Groups: Recurrent pregnancy loss and RIF, the impact of environmental factors on reproductive health, anovulatory infertility and "challenging stimulations," and psychological aspects in infertile couples.

One session is dedicated to the latest developments in the induction of multiple follicular growth—an ever-central aspect of reproductive therapies.

Finally, three keynote lectures are scheduled, featuring members of the International Scientific Committee.

As in previous years, the SIFES 2025 National Congress will give significant attention to presentations from young professionals working in the field of Reproductive Medicine interested in sharing their original work.

In three separate sessions, 31 studies, selected from those submitted and evaluated by a four-member committee, will be presented. The commission will also supervise the selection of winners of SIFES 2025 awards.

This year, the abstracts of the selected oral presentations will be published in the RIVER Journal, the official journal of SIFES-MR and SIERR.

We are proud of this publication!

Paola Anserini
Chair of SIFES-MR

Lucia De Santis
Editor in Chief of River Journal
SIERR Past Chair

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Oral Communications





Sustainability and environmental awareness in reproductive medicine: a call for action

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Introduction

Climate change and loss of biodiversity have been advocated by Lancet as the biggest threat to human health for our century^[1], and this warning involves human reproductive health, too^[2]. It is believed that such threat is caused mostly by anthropogenic GreenHouse Gas (GHG) emissions^[3]. Paradoxically, healthcare systems, whose goal is to promote and guarantee health among citizens, are surely responsible for a part of global carbon footprint^[4]. Recent publications have called for an emergency action to reduce healthcare systems environmental footprint^[5], even in the gynaecological field^[6]. As for reproductive medicine, an important open debate is how to make laboratories truly sustainable^[7], but it is not known yet whether clinicians and embryologist are aware of the significant environmental footprint regarding the care they provide in their everyday clinical practice.

Objectives

To explore the sustainability interest and environmental awareness among fertility staff members and to evaluate whether the internal awareness of appropriateness and sustainability is shared among colleagues from different centers, a survey was sent via email to Humanitas Fertility Center staff and to the SIFES-MR (Italian Society of Fertility, Sterility, and Reproductive Medicine) members.

Results

A total of 87 individuals responded to the survey, up to 451 sent emails (answer rate 19.29%), over a three weeks' period. Even with a such low response rate, up to 64.37% of participants believed that there was avoidable resource waste in their clinical activity, while 28.74% reported an excess of unnecessary diagnostic tests. In this context, 70% of respondents were inclined to adopt more sustainable policies. In fact, a common interest in implementing material recycling (84.00%) emerged, using sterilizable materials (77.46%) and reducing the use of disposable materials (82.76%). Moreover, the analysis showed that demographic and professional factors did not significantly influence opinions on the safety, effectiveness, medico-legal relevance and environmental sustainability of healthcare practices. Regarding the factors that most influence clinical practice decisions, effectiveness and safety were identified as the most important ones (in 91.95% and 93.10%, respectively, of the respondents). Environmental issues, on the other hand, significantly influence clinical practice for 37.93% of respondents.

Conclusion

Healthcare staff showed a strong interest and awareness regarding environmental sustainability, highlighting a willingness to review and modify their clinical practices in response to this need, as well as to adapt and revise corporate policies. This positive attitude provides a solid foundation for the initiation of concrete changes in daily practices, which have so far been only theoretical. An immediately actionable source of GHG emissions could reside in the reduction of inappropriate and avoidable tests, procedures, tools and treatments used in clinical practice. It is evident that the awareness campaigns already carried out have had a positive impact, raising genuine awareness among professionals, who are now motivated to adopt practical measures and translate good intentions into real actions, thus contributing to an evolution of clinical practice towards a more sustainable direction.

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Emotion dysregulation and body dissociation in infertile women: an exploratory study

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Introduction

The relationship between emotion dysregulation and somatoform dissociation was documented in several works on both clinical and non-clinical samples ^[1]. However, despite the literature reporting experiences of disintegration and loss of control over one's body ^[2], no studies investigated the association between these constructs in the specific context of infertility.

Objectives

The aim of this work is to clarify the role of emotion dysregulation in relation to aspects of bodily disconnection involved in assisted reproductive technology procedures, where experiences of somatoform dissociation may arise. The current study recruited 79 women from an Assisted Reproduction Treatment (ART) center at the start of procedures. A socio-demographic questionnaire and two self-report questionnaires were administered: the Difficulties in Emotion Regulation Scale (DERS) for the assessment of emotion dysregulation and the Scale of Body Connection (SBC) for the measure of body disconnection in the two dimensions of dissociation and body awareness. Correlational and regression analyses were conducted to test the predictive role of overall emotional dysregulation on dissociation and body awareness.

Results

Higher levels of emotion dysregulation were positively associated with dissociative processes and negatively with body awareness. Specifically, all DERS subscales and total score correlated with the dissociation subscale of the SBC, while only the total score of non-acceptance, awareness, strategies, and clarity subscales of the DERS correlated with the body awareness subscale of the SBC. Furthermore, overall levels of emotion dysregulation were predictive of high levels of dissociation and low levels of body awareness, explaining, respectively, 40% and 17% of the variance.

Conclusions

Results suggest that difficulties in emotion regulation may be involved in mind-body disconnection processes, which can manifest in women undergoing fertility treatments and may interfere with their ability to attend to their body signals while going through these processes.

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FertileMinds: planting seeds of fertility awareness in Italy

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Introduction

According to the WHO, infertility is increasing, affecting 17.5% people worldwide. Italy exhibits one of the largest disparities between intended and realized fertility in Europe, known as the fertility gap. Despite an average desired family size of approximately two children, Italy's total fertility rate was only 1.20 in 2023. This persistent decline is largely attributed to delayed childbearing, influenced by psychological and socio-economic factors. Simultaneously, there is a lack of awareness regarding behavioral and modifiable factors that may contribute to infertility, compounded by insufficient understanding of when to seek support for reproductive health concerns. Although the International Reproductive Health Education Collaboration (IRHEC) states the importance of fertility and reproductive health education during adolescence, to our knowledge there is no research on this topic in the Italian context.

Objectives

People at the peak of their reproductive age tend to take their fertility for granted: for instance, they often underestimate the age-related decline in fertility and overestimate the success rate of medically assisted reproductive technologies. FertileMinds is a fertility awareness project that adopts a preventive approach, targeting 17-18-year-olds in high schools with the aim of initiating discussions about fertility, raising awareness on medical, psychological and social aspects surrounding reproductive health and the risk factors involved. The project consisted of six three-hour sessions covering topics such as: fertility myths, lifestyle risks and protective factors, stress, infertility stigma, fertility preservation in case of oncological pathologies and social freezing, generative paths (ART, adoption, heterologous fertilisation, childfree). The FertileMinds project aims to promote fertility awareness while respecting each student's reproductive autonomy, providing evidence-based information and encouraging creative activities, in order to stimulate both emotional and rational learning.

Results

Students demonstrated a significant improvement in their knowledge on reproductive health, particularly in differentiating the impact of different risk factors (e.g. older age, strenuous exercise, underweight and overweight, sexually transmitted diseases). They also developed a better understanding of the medical and psychological aspects of infertility, as well as the social stigma often associated with it. Furthermore, students have also increased their awareness on Assisted Reproductive Technologies (ART).

Conclusions

Adolescence, compared to adulthood, allows a more open and receptive approach to this topic, due to the relative lack of social pressure on becoming parents at this stage. Young people are willing and open to acquire such knowledge, particularly when approached in a non-paternalistic, respectful and inclusive manner.

Recommended reading

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The #Enzian classification: is it time to rethink the impact of endometriosis on IVF?

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Introduction

Although the impact of endometriosis on OS has been widely studied, the results are not conclusive. Most studies have focused on the impact of endometriomas rather than endometriosis. The possibility of diagnosing pelvic endometriosis in a detailed and non-invasive manner has opened up new scenarios in assessing the impact of this condition on fertility and IVF. Several indices have been developed to predict the impact of endometriosis on natural fertility, such as EFI or rASRM. To date, no score has ever been evaluated to predict the IVF outcomes in patients with endometriosis.

Objectives

A retrospective cohort study was conducted at our IVF Unit between January 2023 and December 2024. The study included 61 women with at least one ultrasound feature of endometriosis who underwent ovarian stimulation for IVF. All women underwent ultrasound examination before ovarian stimulation: the pelvis was examined in the anterior, lateral and posterior compartments and deep infiltrating endometriotic lesions were considered for this study. All lesions were classified according to the #Enzian classification. The primary endpoint is the number of eggs retrieved. Poor oocyte recovery was defined as a number of oocytes collected of less than 4. Secondary endpoints are: number of metaphase II oocytes; number of fertilized oocytes; number of blastocysts and cleaved embryos obtained. Blastulation rate and fertilization rate were also evaluated. The impact of #Enzian classification was assessed by univariate logistic regression model. Odds Ratios (OR) are displayed with two-sided 95% confidence interval.

Results

The mean age of the population was 37 ± 3.2 years. Mean BMI and serum AMH levels were 22.42 ± 2.5 kg/m² and 2.26 ± 3.34 ng/ml, respectively. According to the #Enzian classification, 26 patients (43%) had lesions classified in compartment O, 18 (29.5%) in compartment A, 47 (77%) in compartment B, and 10 (16%) in compartment C. Twelve patients (20%) had previously undergone surgery for endometriosis: 12 patients (20%) underwent ovarian surgery, while 8 (13%) had deep endometriosis eradication surgery. In the univariate analysis, endometriosis of compartment C was associated with poor oocyte yield (OR: 8.33; 95% CI: 1.13-69.7; p-value <0.02). Endometriosis of compartments A, B and O was not correlated with poor oocyte yield. After adjusting for the effect of female age, endometriosis of compartment C was still significantly associated with low oocyte recovery (adjusted OR: 8.32, 95% CI: 1.13-69.6, p-value <0.03). Moreover, endometriosis of compartment O was associated with no blastocyst development (OR: 5.99; 95% CI: 1.52-30.4; p-value <0.01). After adjusting for the effect of female age, endometriosis of compartment C was still significantly associated with no blastocyst development (adjusted OR: 5.7, 95% CI: 1.46-58.9, p-value <0.04).

Conclusions

In clinical practice, correct characterization and classification of endometriosis by ultrasound can help predict poor oocyte recovery and no blastocyst development. Further investigations are needed to better understand the mechanisms that extra-ovarian endometriosis has on oocyte recovery. This study is inherently limited by its retrospective design and relatively small sample size.

Recommended reading

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Heterogeneity of adenomyosis in different population

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Objectives

Adenomyosis is increasingly recognized as a potential cause of infertility, yet its precise impact on reproductive outcomes remains unclear. This study investigated the correlation of adenomyosis localization and infertility, highlighting its effects on endometrial receptivity, implantation failure, and pregnancy outcomes. Understanding this correlation may improve diagnostic and therapeutic approaches in reproductive medicine. The main aim of this study was to evaluate the association of the precise localization of adenomyosis (internal myometrium and/or external myometrium) and the reproductive outcomes in different populations composed of infertile patients, Recurrent Pregnancy Loss (RPL) and patients with at least one Live Birth (LB).

Methods

This retrospective observational study, included infertile patients admitted to Gynecological Ultrasound Unit of the University of Rome "Tor Vergata", Italy, between June 2018 and February 2025. All patients underwent 2D, 3D and Power-Doppler (PD) Trans Vaginal Sonography (TVS) examination in order to evaluate accurately the presence and the localization of adenomyosis (internal and/or external myometrium). The diagnosis of adenomyosis was performed with the presence of at least two direct signs based on current Morphological Uterus Sonographic Assessment (MUSA) classification. The ultrasonographic diagnosis was performed before the pregnancy (LB group), before the miscarriage (RPL group) and before the IVF treatment (infertile group). All patients with adenomyosis and infertility performed in-vitro fertilization after at least 12 months of regular, unprotected sexual intercourse were included.

Results

Among 383 patients with infertility and adenomyosis, 223 were infertile, 80 experienced RPL and 80 achieved at least one live birth (LB). Among these groups the different position of adenomyosis was investigated dividing in only inner myometrial, only external and mixed. A statistically higher presence of only inner myometrium, diffuse, moderate and severe adenomyosis was described in the group with infertility compared to LB group ($p=0.0486$; $p<0.0001$; $p=0.0282$; $p=0.0016$, respectively). The presence of only adenomyosis was significantly higher in the patients with RPL compared to infertile ones ($p<0.0001$), while the presence of endometriosis, specifically DIE ($p<0.0001$), diffuse ($p<0.0001$), moderate ($p=0.0005$) and severe ($p=0.0016$) adenomyosis were significantly higher in the infertile population compared to RPL (Table 1 and 2).

Conclusions

Adenomyosis is linked to poor reproductive outcomes through mechanisms that remain incompletely understood. Our study demonstrated a strong association of the presence of adenomyosis in the RPL and infertile group compared to LB group. In particular, the inner myometrial involvement was strongly presented in the infertility group.



Heterogeneity of adenomyosis in different population

Population characteristics	Infertility 223	RPL 80	p value
Mean age at diagnosis	39.7 ± 6.1	36.5 ± 5.6	
BMI	20.45 ± 5.2	23.28 ± 4.5	
Only adenomyosis	92 (41.3%)	56 (70%)	< 0.0001
Adenomyosis+endometriosis	131 (58.7%)	24 (30%)	< 0.0001
Endometriosis	131 (58.7%)	24 (30%)	< 0.0001
- DIE	121 (54.3%)	18 (22.5%)	< 0.0001
- endometriomas	10 (4.5%)	6 (7.5%)	NS
Adenomyosis			
- only inner myometrium	44 (19.7%)	22 (27.5%)	NS
- only outer myometrium	111 (49.8%)	32 (40%)	NS
- mix	63 (28.3%)	26 (32.5%)	NS
- focal	51 (22.9%)	24 (30%)	NS
- diffuse	171 (76.7%)	30 (37.5%)	< 0.0001
- mild	90 (40.4%)	62 (77.5%)	< 0.0001
- moderate	80 (35.9%)	12 (15%)	0.0005
- severe	53 (23.8%)	6 (7.5%)	0.0016

Table 1. Description of the different localization of adenomyosis in the group with infertility compared to RPL (Recurrent Pregnancy Loss). BMI: Body Mass Index; DIE: Deep Infiltrated Endometriosis.

Population characteristics	Infertility 223	LB 80	p value
Mean age at diagnosis	39.7 ± 6.1	35.0 ± 6.3	
BMI	20.45 ± 5.2	21.45 ± 5.2	
Only adenomyosis	92 (41.3%)	18 (22.5%)	0.0028
Adenomyosis+endometriosis	131 (58.7%)	62 (77.5%)	0.0028
Endometriosis	131 (58.7%)	62 (77.5%)	0.0028
- DIE	121 (54.3%)	46 (57.5%)	NS
- endometriomas	10 (4.5%)	4 (5%)	NS
Adenomyosis			
- only inner myometrium	44 (19.7%)	8 (10%)	0.0486
- only outer myometrium	111 (49.8%)	52 (65%)	0.0195
- mix	63 (28.3%)	20 (25%)	NS
- focal	51 (22.9%)	20 (25%)	NS
- diffuse	171 (76.7%)	40 (50%)	< 0.0001
- mild	90 (40.4%)	56 (70%)	< 0.0001
- moderate	80 (35.9%)	18 (22.5%)	0.0282
- severe	53 (23.8%)	6 (7.5%)	0.0016

Table 2. Description of the different localization of adenomyosis in the group with infertility compared to LB (Live Birth). BMI: Body Mass Index; DIE: Deep Infiltrated Endometriosis.



Genetic variation is higher on sperm than blood regardless male age: implications for preconceptional genetic screening

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Introduction

Advanced Paternal Age (APA) is associated with an increased risk of adverse health effects in children apparently caused by an increase in de novo mutations on spermatozoa due to the spermatogenesis process, where constant cell division occurs, and mutations in the germ line that may not appear in the somatic line accumulate. Germ cell mosaicism then is a matter of concern, of relevance on the prevention of future children's disease, since preconceptional tests are carried out on blood instead of spermatozoa, potentially causing false negatives if the mutation is present in spermatozoa but not on the analyzed tissue.

Objectives

To demonstrate the different genetic alterations in spermatozoa by showing the difference in the DNA sequence between blood and sperm and how this difference increased with age to develop a diagnostic test directed on spermatozoa for the prediction of disease in offspring.

Results

When compared the numbers of genetic variations for all patients independently of age in both tissues, we observed a statistically significant increase (0.86%) in spermatozoa (28188.76 ± 693.76 , IC95% 27063-33441) in comparison with blood (27948.28 ± 653.60 , IC95% 25941-33470) ($P=1.57 \times 10^{-15}$).

Furthermore, we analyzed the number of variations considering the age of patients. In patients ≤ 30 years old we observed a statistically significant increase (0.77%) in spermatozoa (28154.94 ± 425.44) in comparison with blood (27938.08 ± 327.10) ($P=1.44 \times 10^{-7}$).

Equally, we observed an increase (0.93%) of number of variations in spermatozoa (28218.81 ± 781.23) in comparison with blood (27957.35 ± 843.59) ($P=2.79 \times 10^{-9}$) in patients ≥ 45 years old. However, when analyzed the difference of the number of genetic variations between spermatozoa and blood of patients ≤ 30 and patients of ≥ 45 years old no statistically significant difference was observed ($P=0.1$).

Moreover, when we compared the genetic variations between spermatozoa and blood, there were 26996 common variants between both tissues, but interestingly we observed tissue specific variants, only presented in sperm or blood. We observed $876.96 \pm$ variants only in the spermatozoa and 635.47 ± 111.39 variants only in the blood.

Conclusions

In this study, we show that there is a major number of genetic variations in sperm than in blood. However, considering the increased numbers of genetic variations of different male's ages, there are not statistically significant differences.

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LH priming before ovarian stimulation in poor responders: a self-controlled study comparing long protocol with LH priming vs. antagonist protocol

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Introduction

Poor Ovarian Response (POR) is a major challenge in IVF, particularly in women with Advanced Maternal Age (AMA), where Diminished Ovarian Reserve (DOR) and oocyte quality loss reduce success rates [1,2]. The POSEIDON classification highlights the need for tailored strategies, especially for POSEIDON-4 patients, who represent the majority of POR cases. Previous studies suggest that the long protocol with LH priming yields better outcomes than the antagonist protocol in poor responders, but further validation is required [3]. Given the potential of LH priming to enhance follicular recruitment, this study compares both protocols within the same patients to assess improvements in ovarian response and clinical outcomes.

Aims

The primary objective of this study was to evaluate the efficacy of the long protocol with a seven-day LH priming regimen under GnRH agonist downregulation in poor responders. The study assessed key outcomes, including the total number of oocytes retrieved, metaphase II (MII) oocytes, fertilization rates, viable embryo yield, cycle interruption rates, and clinical pregnancy rates. Forty-five women with poor ovarian response served as their own controls, first undergoing an ovarian stimulation cycle with an antagonist protocol, which failed, and subsequently repeating the cycle using a long protocol with a seven-day LH priming regimen under GnRH agonist downregulation.

Results

The long protocol with LH priming demonstrated a significant improvement in ovarian response compared to the antagonist protocol. It resulted in a higher number of total and MII oocytes, improved fertilization rates, and an increased number of viable embryos transferred. Cycle interruption rates were notably lower with the long protocol (28.89% vs. 46.67% in the antagonist protocol). Additionally, clinical pregnancy rates were significantly higher, with 12 pregnancies in the LH priming group versus 3 in the antagonist group. One pregnancy occurred in a patient who had failed to reach embryo transfer in the antagonist cycle, emphasizing the protocol's potential for rescuing poor responders.

Conclusions

The long protocol with LH priming significantly improved ovarian stimulation outcomes in poor responders, enhancing both oocyte quantity and quality. The reduced cycle discontinuation rate indicates a more effective stimulation approach. These findings support the use of LH priming in clinical practice, though further prospective studies are necessary to refine patient selection and optimize treatment strategies.

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Multiple sclerosis and infertility: is there any relationship?

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Introduction

With the introduction of Disease-Modifying Therapies (DMTs), patients affected by Multiple Sclerosis (MS) no longer have to abandon their reproductive plans. However, the relationship between MS and infertility remains unclear. It is still uncertain whether the prevalence of infertility in MS patients is comparable to that of the general population or if it is higher^[1]. Several factors may confound the relationship between MS and infertility, including ongoing treatments (some of which are teratogenic), disease progression, and patients' concerns about pregnancy. While limited studies suggest no significant difference in infertility prevalence between MS patients and the general population^[2], available data are scarce and often conflicting. Time To Pregnancy (TTP) is a key measure of fecundity and is particularly relevant for MS patients, who may have a narrow window for conception due to treatment intervals.

Objectives

This study aims to evaluate the time to pregnancy in couples where at least one partner has MS, the incidence of infertility, pregnancy rates achieved through Assisted Reproductive Technologies (ART), and pregnancy outcomes in terms of live births. In 2011, we established a multidisciplinary protocol in collaboration with the Multiple Sclerosis Unit. All patients (both male and female) planning to conceive were offered a consultation with a reproductive specialist to assess fertility status and address any subfertility factors. Couples in which one partner had MS were prospectively followed from referral by neurologists until conception and delivery. Collected data included baseline patient characteristics (age, Anti-Müllerian Hormone [AMH] levels, semen parameters, ultrasound findings), time to pregnancy, conception method (spontaneous or ART), and pregnancy outcomes.

Results

A total of 71 couples underwent preconceptional assessment before attempting pregnancy. The affected partner was female in most cases, with only two couples having a male partner with MS. A 12-month follow-up was completed for 57 couples; 11 couples (15.4%) were lost to follow-up, one couple postponed pregnancy due to an MS relapse, and two couples were still within their first year of attempts. Among the 57 couples with at least 12 months of follow-up, 33 achieved at least one spontaneous pregnancy (57.9%), resulting in a total of 45 spontaneous pregnancies. The mean TTP was 5.3 months (± 6.2). The remaining 24 couples (42.1%) did not achieve a spontaneous pregnancy within 12 months and were classified as infertile. Among them, 20 opted for ART treatment, with 7 achieving pregnancy (35%), while four couples were still undergoing ART at the time of analysis. Four couples conceived spontaneously after more than 12 months of attempts. The mean age at conception was 32.9 years (± 4.6) for those who conceived spontaneously and 36.7 years (± 5.8) for those who conceived through ART. Among the 45 spontaneous pregnancies, 35 resulted in the birth of healthy full-term infants (77.7%), nine ended in first-trimester miscarriage, and one was terminated due to worsening neurological condition. Among the 7 pregnancies achieved via ART, five resulted in healthy full-term births (71.4%), while one ended in miscarriage and one in ectopic pregnancy. Regarding ongoing DMT use, therapy was continued in 31.1% of spontaneous pregnancies and 14.3% of ART pregnancies, mostly with Natalizumab.

Conclusions

This prospective observational study highlights a relatively high percentage of MS-affected couples who do not conceive spontaneously within 12 months of unprotected intercourse. However, due to the limited sample size, we cannot conclude with certainty that infertility rates are higher in this population compared to the general population, particularly as maternal age was a major factor influencing TTP. Additionally, the increasing use of Natalizumab in young MS patients may reduce the need for pregnancy delays related to DMT washout, which was one of the main reasons to postpone pregnancy in the last decades. These findings bring attention to the importance of implementing multidisciplinary protocols to assess fertility in MS patients early in their reproductive planning. Timely evaluation and, when necessary, access to ART may help reduce time to pregnancy while minimizing disease-related complications.

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Fertility preservation and oocytes quality in ovarian cancer

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Introduction

To improve the quality of life in oncological patients, Fertility Preservation (FP) should be considered part of cancer treatment. Approximately 5400 cases of Ovarian Cancer (OC) are recorded in Italy in 2024 and it is one of the cancers that most negatively impacts fertility in young women. Currently, cryopreservation of Mature Oocyte (MO) is the standard technique for FP. However, studies on ovarian response to Controlled Ovarian Hyperstimulation (COH) in cancer patients remain limited. A few reports suggest that OC may negatively affect follicular development and oocyte quality, but the mechanism remains unclear. A retrospective single-center case-control study was conducted in the *In Vitro* Fertilization (IVF) Unit at the Sandro Pertini Hospital in Rome between 2016 and 2024. A total of 111 women were enrolled in the study, including 49 with OC and 62 infertile patients (male or tubal factor infertility), matched for demographics and type of trigger for oocyte induction (GnRH agonist).

Aims

The aim of this study was to assess the influence of OC on ovarian response in terms of total MO, percentage of Immature Oocytes (IO) and Abnormal Oocytes (AO) retrieved in these oncological patients compared to not oncological patients undergoing COH for IVF for male or tubal factor infertility. The primary outcome was number and quality of oocytes retrieved. Statistical analysis was performed using the t-test for normally distributed variables and the Wilcoxon test for non-normally distributed variables to assess group differences.

Results

The demographic characteristics in the group of oncological patients were: median age 26.1 years, median BMI 22.6, median serum AMH level 1.51 ng/ml, while those in the control group were: median age 32.4 years, median BMI 21.1, median serum AMH level 3.95 ng/ml. The analysis revealed that OC patients required higher gonadotrophin doses than control group (2700 U vs 1350 U, $p < 0.001$) even though the median length of stimulation did not show statistically significant differences (12 vs 11., $p = 0.353$). There were no statistically significant differences in terms of total retrieved oocytes (10.1 vs 11.5, $p = 0.265$), total MO (7 vs. 8, $p = 0.177$), IO (0 vs 1, $p = 0.401$) and total AO (0 vs 1, $p = 0.689$). In conclusion, the diagnosis of OC does not seem to be associated with an impairment of the ovarian response to COH in term of total MO retrieved, AO and IO.

Conclusions

Although this condition affects the ovary, the OC diagnosis does not appear to be a risk factor in obtaining fewer mature, immature or abnormal oocytes. Further studies are necessary to evaluate the etiopathogenetic mechanisms underlying oocyte abnormalities in this specific group of female oncology patients. The study has limitations, including its retrospective design, small sample size and inability to match groups by age, because IVF patients for OC are usually younger and by serum AMH level, because at the time of FP, the majority of patients in the oncological group had already undergone ovarian surgery.

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Laparoscopic transplantation of cryopreserved ovarian tissue with autologous platelet-rich plasma in oncological patients: a pilot study

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Introduction

Ovarian tissue auto-transplantation is an established fertility preservation method in restoring endocrine function and fertility. The most crucial time during which 70-80% of follicles are lost in the graft is the transplantation time itself, mainly due to a perioperative ischemic window. Autologous Platelet-Rich Plasma (PRP) is a method involving the extraction and concentration of a patient's own platelets through the centrifugation of a peripheral blood sample. This process is believed to trigger platelet degranulation, leading to the release of growth factors that can enhance blood vessel formation. This is a pilot, interventional, cohort study conducted on five consecutive patients, who cryopreserved ovarian tissue before chemo(radio)therapy for oncologic diseases and were scheduled for orthotopic reimplantation with the use of autologous PRP to treat iatrogenic premature ovarian failure, from March to December 2024. Autotransplantation was performed by standard four-port laparoscopy, creating pockets in the ovarian cortex and/or peritoneal cavity to insert ovarian tissue fragments covered by gelled PRP (10 mL). Pre-, intra- and post-operative data were collected for each patient. Restoration of endocrine ovarian function was assessed monthly after surgery through menstrual diary.

Objectives

Primary outcome was the rate of adverse reaction to PRP use, rate of intra- and post-operative surgical complication. Secondary outcome was the rate of restored ovarian function and median (absolute range) time interval to restore normal ovarian function.

Results

The mean age ($\pm$ Standard Deviation, SD) at ovarian tissue retrieval and transplantation was 27,6 ($\pm$ 5,7) and 37,2 ($\pm$ 5,8) years, respectively. During study period, no intra- and post-operative complications nor adverse reaction to PRP use was observed. All patients experienced resumption of regular ovarian function after a median (range) period of 3 (1–6) months. One patient achieved spontaneous pregnancy 3 months from surgery.

Conclusions

The use of autologous PRP during orthotopic reimplantation of cryopreserved ovarian tissue seems to be a feasible and safe option to treat POI related to past gonadotoxic therapy. Further data are needed to evaluate its efficacy in terms of graft longevity and reproductive outcomes compared to a matched historical cohort

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Ovofast: preliminary results of a successful application of a shortened warming protocol for oocytes cryopreserved using standard vitrification, while the ultra-shortened protocol showed cytoskeletal alterations

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Introduction

Recent studies on Shortened oocyte Warming (SW) protocols have shown promising results, with survival rates and developmental competence comparable to or even superior to the standard protocol. These methods improve IVF efficiency by minimizing exposure to suboptimal temperatures, reducing osmolarity-induced stress, and mitigating the harmful effects of cryoprotectants [1]. However, research on SW protocols has predominantly focused on oocytes vitrified using Rapid Vitrification (RV) techniques, while data on their effectiveness for oocytes cryopreserved with Standard Vitrification (SV) remains limited. With millions of oocytes already vitrified using conventional methods, further research is needed to evaluate the effectiveness of SW protocols for these oocytes.

Objectives

To evaluate the efficiency of SW and Ultra-Shortened Warming (USW) protocols for oocytes cryopreserved using SV.

Results

This prospective sibling study included 390 mature human oocytes. Experiment-1 compared the standard warming (Group A) and the SW protocols (Group B), and Experiment-2 compared the SW and USW protocols (Group C). Sub-analyses focused on spindle/chromosomal configurations and oocyte quality scores (range:0-10) using an Artificial Intelligence (AI) model. Group A followed the standard warming protocol (10-minutes), Group B the SW protocol (1-minute in 1.0 M trehalose, and 1-minute in 0.5 M trehalose at 37°C), and Group C the USW protocol (1-minute in 1.0 M trehalose at 37°C). Survival rates were evaluated two hours post-warming. Spindle configurations were classified as normal, slightly aberrant, or aberrant, while chromosomal distributions as normal, misaligned, or displaced. In Experiment-1 (Group A n=150 oocytes, Group B n=148 oocytes) no significant differences were observed in survival rates (Group A:89.33% vs. Group B:93.24%, p=0.23), chromosomal [Group A (n=21): normal 72%, misaligned 14%, displaced 14% vs. Group B (n=21): normal 67%, misaligned 19%, displaced 14%, p=0.73] and spindle configurations [Group A (n=13): normal 85%, aberrant 15% vs. Group B (n=8): normal 75%, aberrant 25%, p=0.58], or AI-derived quality scores (n=57, Group A: 5.88±2.41 vs. Group B: 5.58±2.42, p=0.67). The SW protocol reduced workflow time by 80%. In Experiment-2 (Group B n=46 oocytes, Group C n=46 oocytes) no significant differences were observed in survival rates (Group B: 93.47% vs. Group C: 97.82%, p=0.30) and AI-derived quality scores (n=22, Group B: 4.58±2.84 vs. Group C: 4.85±2.66, p=0.79). We found significant differences in chromosomal [Group B (n=14): normal 64%, misaligned 14%, displaced 21% vs. Group C (n=16): normal 13%, misaligned 25%, displaced 63%, p=0.003] and spindle configurations [Group B (n=14): normal 79%, slightly aberrant 7%, aberrant 14% vs. Group C (n=14): normal 14%, slightly aberrant 14%, aberrant 72%, p<0.001].

Conclusions

Our results highlight the potential of the SW protocol, maintaining efficiency comparable to standard methods while reducing workflow time and minimizing exposure to suboptimal conditions. In contrast, the USW protocol demonstrated similar survival rates but it induced cytoskeletal alterations, particularly in chromosome and spindle configurations. If confirmed by independent studies on oocytes cryopreserved using RV, this protocol could demonstrate its universal applicability.

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A tool based on artificial intelligence for the evaluation of fresh donor oocytes and the prediction of blastulation: a prospective investigation

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Introduction

In recent years with the increase of the maternal age, many couples decide to start IVF treatments with donated oocytes. However, despite the fertility of donors, not all oocytes possess the capacity to develop properly, with many failing to reach the blastocyst stage. Visual assessment of oocyte morphology remains a prevalent practice, but it is subject to limitations, being subjective and ineffective in predicting competence. Recent advancements in Artificial Intelligence (AI) have emerged as a promising solution to standardize oocytes' analysis, thereby opening new perspectives in predicting their development potential. In this context, the AI-Based Image analysis system, which gives a score as a result, may enhance the management of treatments involving donated oocytes. The Score provided by the AI-Based Tool is represented by a number between 0 and 10, where 0 is associated with the lowest morphologic quality while 10 with the highest.

Objectives

This retrospective analysis has as purpose to evaluate the prediction capacity of the AI tool by determining the association between the AI-Based Score and the blastulation rate of fresh donated oocytes. The implication of the AI in the oocyte donation may help IVF clinics by assisting in the assignment of a suitable number of eggs to each couple, eliminating unnecessary overproduction of blastocysts.

Results

The analysis was conducted at 3 IVF clinics in Spain (Ginefiv Barcelona, Ginefiv Madrid, Ginemed Seville) on 1275 MII-oocytes' images collected from 145 donors and distributed to 171 recipient couples. The images were taken after oocytes' denudation and before ICSI, observed under optical microscopy, along with documented fertilization and embryo development outcomes up to the blastocyst stage, was compiled. The 7.5% of the oocytes considered (N=96/1275) were not assessed because of image defects (blurred images, CC surrounding the oocyte, dark images, stripes). Granularity, abnormal shape and refractile bodies were significantly associated with lower AI-Based Score. 2PN fertilization (OR:1.08) and blastocyst formation (OR:1.19) had a significant correlation with the AI-Based Score, even after adjusting with donor age, sperm motility, and culture medium (AUC:0.646). The AI software showed a tendency to overestimate the number of blastocysts obtained in 43% of cycles. In 82% of the cases the number of blastocysts obtained was within the predicted range or was higher (N=140), while in 18% (N=31) it was lower. The predicted probability of achieving ≥ 1 live birth was significantly associated with the actual cumulative live birth rate (OR:1.55 per 10% increase; AUC:0.691). In 6% of cases no blastocysts were obtained in the IVF cycles while the tool predicted from 2 to 8.

Conclusions

As demonstrated in this study, an AI-based evaluation of donor oocyte quality correlates with blastocyst formation and cumulative live birth outcomes in a multicenter cohort.

The findings indicate that the integration of AI in IVF cycles may provide an advantage in the objectivity and standardisation of the clinical management process. In addition, the incorporation of supplementary data and confounding factors has the potential to yield future enhancements to the prediction model.

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Intracytoplasmic injection of testicular sperm: blastulation rate in patients with obstructive azoospermia, non-obstructive azoospermia, and cryptozoospermia

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Introduction

This study examines the impact of testicular sperm origin on embryonic development in patients with Obstructive Azoospermia (OA), Non-Obstructive Azoospermia (NOA), and cryptozoospermia undergoing ICSI with TESE. A total of 874 cycles were analyzed, with patients stratified by diagnosis and female partner's age (<35 and ≥35 years). Evaluated parameters, such as blastulation rates and blastocyst expansion grades, revealed significant differences that correlate with the severity of spermatogenic defects.

Aims

Patients were categorized into three groups—obstructive azoospermia (368 cycles), non-obstructive azoospermia (253 cycles), and cryptozoospermia (253 cycles)—and further stratified by the female partner's age (<35 or ≥35 years). Semen and testicular tissues were processed according to WHO 2010 guidelines, with retrieved oocytes evaluated based on their morphology and maturity, while blastocysts were assessed for quality and expansion using the Istanbul Consensus criteria. Data were analyzed using ANOVA and Tukey's test to compare the groups.

Results

Blastulation rates were significantly higher in the OA group compared to NOA and cryptozoospermia ($p < 0.0001$ for women <35 years and $p = 0.002$ for women ≥35 years). The cryptozoospermia group exhibited delayed embryonic development, reduced hatching blastocysts, and overall lower embryo quality. While female age influenced ovarian reserve, it did not result in significant variations in blastulation rates among the analyzed subgroups.

Conclusions

The study reveals a significant paternal effect on embryonic development. This effect is closely linked to the severity of spermatogenic defects. Blastulation was notably more efficient in OA patients. This efficiency underscores the pivotal role of sperm quality in achieving embryonic competence. Conversely, cryptozoospermia patients showed limitations in embryo quality when using testicular sperm. These limitations suggest that post-testicular defects and impaired spermatogenesis adversely affect outcomes. The OA group achieved the best ratio of retrieved oocytes to fertilized oocytes and blastocysts. Surgical sperm retrieval proved highly effective for this group. However, factors such as oocyte quality and culture conditions also significantly influence outcomes. Future research should refine culture protocols and incorporate maternal contributions to better understand assisted reproduction success.

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The validity of the OPN fertilization pattern

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Background

The evaluation of zygotes with non-standard ProNuclear (PN) patterns, such as OPN, 1PN, and 3PN, along with other rare variations, has gained increasing interest as a strategy to optimize embryo availability and maximize cumulative clinical success per cycle⁽¹⁾. However, this approach raises both clinical and ethical concerns, particularly regarding the controversial hypothesis that OPN zygotes may develop through pre- and post-implantation stages without pronuclear formation. Time-Lapse Technology (TLT) serves as a valuable tool in addressing these uncertainties by enabling continuous and precise monitoring of embryo morphokinetics⁽²⁾.

Aims

This study aims to determine whether the OPN fertilization pattern, assessed through static observation within the 16–18 Hours Post-Insemination (HPI) window, permits embryo development in the absence of conventional pronuclear formation.

Results

This retrospective study included patients who underwent ovarian stimulation (GnRH antagonist protocol with hCG/agonist trigger) between 2013 and 2020, yielding a total of 6052 oocytes. All oocytes were inseminated via ICSI and cultured in a TLT incubator. The ICSI cycles resulted in 5059 zygotes displaying at least one PN, 249 degenerated oocytes, and 744 zygotes classified as true OPN. Embryo development was monitored until the blastocyst stage.

Morphokinetic analysis revealed critical associations between pronuclear behavior and blastocyst development. Among 4478 2PN zygotes, those in which PN fading occurred between 18–20 hpi exhibited the highest blastocyst formation rate (<16 hpi: N=10, 40.0%; 16–18 hpi: N=44, 47.7%; 18–20 hpi: N=309, 65.4%; >20 hpi: N=4115, 53.4%; $p<0.001$). Conversely, in 87 cases where PN formation was delayed beyond 16 hpi, blastocyst formation was significantly reduced (54.5% vs. 31%; $p<0.001$).

Among the 744 true OPN zygotes, 94 (13%) underwent at least one cleavage event; however, none progressed beyond the compaction stage (cell count: mean $\pm$ SD 3.1 $\pm$ 1.6, median 3, range 1–10). The majority exhibited abnormal cleavage patterns, including direct, reverse, and chaotic divisions (N=71, 76%), while others displayed significant vacuolization (N=15, 16%) and/or extensive fragmentation (N=36, 38%). Statistical regression analysis, adjusted for confounders such as maternal age, male factor, and PN type, confirmed these associations through Generalized Estimating Equations (GEE).

Conclusions

A small proportion of zygotes capable of reaching the blastocyst stage exhibit pronuclear formation beyond the conventional 16–18 hpi window. In the rare cases where cleavage occurs without pronuclear formation, development invariably ceases at an early stage. The application of TLT highlights the limitations of traditional static observation, which fails to detect a significant number of zygotes, with implications for fertilization assessment, blastocyst prediction, and the utilization of zygotes with atypical pronuclear patterns. Furthermore, TLT provides strong evidence against the hypothesis that blastocyst formation can occur in the absence of pronuclei. Independent validation of these findings is warranted, along with further investigations into fertilization morphokinetics in conventional IVF zygotes.

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Semen quality in patients with hematologic malignancies: a retrospective monocentric study

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Introduction

The hypothalamic-pituitary-testis axis is dysregulated in patients with hematological malignancies. However, data regarding the association between different types of hematological malignancies and semen quality are discordant. While in patients with leukemias a clear deterioration of semen quality is observed, studies on Hodgkin and non-Hodgkin lymphomas have shown that spermatogenesis is not always compromised, and affected patients may present normospermia before treatment.

Aims

The aim of the present study was to assess semen parameters in males affected by hematological malignancies and compare them with a non-cancer population. A retrospective monocentric study was conducted, including patients with hematological malignancies who underwent fertility preservation, at the Maternal and Child Department, Gynecology and Obstetrics, Oncofertility Unit, Federico II University of Naples, from January 2017 to December 2024. A total of 248 patients affected by hematological malignancies and 63 non-cancer males, who underwent in vitro fertilization for female tubal factor selected as the control group, were included. Semen parameters were also compared among the subgroups of Hodgkin Lymphoma (HL), Non-Hodgkin Lymphoma (NHL), and Leukemia (L).

Results

Semen analysis, according to World Health Organization 2021 criteria, showed that the semen parameters of patients with hematological malignancies were at the 25th percentile, except for motility, which was below the 5th percentile. Results showed significant differences in sperm concentration/mL, total sperm number, % of total motility between hematologic malignancies group and control group ($p=0.0004$; $p=0.0003$; $p<0.0001$). Moreover, based on different classification, hematologic malignancies were grouped in 159 Hodgkin lymphoma, 54 non-Hodgkin lymphoma and 35 leukemia and significant differences were found in concentration/mL and total sperm number only between Hodgkin lymphoma and control group ($p=0.004$; $p=0.001$). Instead, the % of total motility appeared statistically significant decreased in all subtypes of hematologic malignancies vs control especially in Leukemia group (HL $p=0.001$; NHL $p=0.006$, L $p<0.001$).

Conclusion

The presented data it has been demonstrated a negative effect of hematological malignancies in semen quality, particularly motility. These results underscore the importance of early fertility preservation strategies in clinical practice and highlight the need for further research to better understand the underlying mechanisms and optimize reproductive outcomes for affected individuals.

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Exploring semen quality among cancer patients

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Introduction

Anticancer treatments (surgery, radiotherapy, chemotherapy) may affect male fertility transiently or permanently ^[1]. Evidence suggests that the disease process itself may affect a man's fertility by influencing spermatogenesis ^[2]. Different study compared semen parameters of oncological patients who performed semen cryopreservation before oncological treatments with healthy men ^[3-7], some studies compared semen samples from patients affected by different types of tumors with conflicting results ^[8-21].

Objective

This retrospective cohort study aimed to assess the semen quality of cancer patients who performed semen cryopreservation at our center from 2014 to 2024 before gonadotoxic treatments.

Results

We collected data of 548 men of which 246 were affected by testicular cancer (45%), 203 by hematological cancer (37%) and 99 by solid cancer (18%). The most frequent types of hematological cancers were Hodgkin lymphoma (39%), non-Hodgkin lymphoma (16%) and leukemia (12%). Among solid cancers the most frequent were gastro-intestinal tumors (27%), tumors of nervous system (18%), sarcoma (11%), head and neck cancer (7%), and osteosarcoma (6%). Semen analysis was performed according to World Health Organization guidelines. Statistical analysis was performed by one-way ANOVA, analysis of covariance was performed by ANCOVA. Patients' age, sperm concentration and total sperm count differed among groups ($p < 0.05$), while volume, total motility and progressive motility did not differ among groups. Adjusting data for age sperm concentration and total sperm count differed among groups ($p < 0.001$). Patients with a testicular cancer had an impaired semen quality at diagnosis, in particular we observed a significant reduced sperm concentration and total sperm count respect to hematological ($p < 0.05$; $p < 0.05$, respectively) and solid tumor ($p < 0.05$; $p < 0.05$, respectively) patients. A sub-analysis was performed comparing semen samples of patients with testicular tumors who performed cryopreservation before ($N=62$) or after ($N=72$) orchiectomy. We did not observe any significant difference in semen parameters in samples cryopreserved before or after orchiectomy.

Conclusions

Our data confirmed that testicular cancer patients had a reduced sperm quality respect to solid and hematological cancer patients. It is reasonable that spermatogenesis impairment is likely due to local (i.e., paracrine factors, anatomic alteration of testicular tissue and hyperthermia due to inflammation) and systemic effects of tumor. In testicular cancer patients, semen cryopreservation before or after surgery offers the same chances of success and should always be performed.

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Proposal for a new diagnostic-therapeutic classification of male factor infertility: preliminary analysis

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Background

Couple infertility is a clinical condition defined as the inability to conceive after at least 12 months of regular and unprotected sexual intercourse. It is estimated that the Male Factor of Infertility (MFI) is present, alone or in combination with the female factor, in about 2/3 of cases of couple infertility. The complete diagnostic pathway of MFI (medical history, physical examination, hormonal tests, scrotal color Doppler ultrasound, complete seminal analysis, possible second level tests such as sperm culture or transrectal prostate ultrasound or genetic tests) allows to categorize the causes of MFI into specific categories that help the clinician to orient himself towards the most appropriate therapeutic choice. These categories have recently been proposed by a group of authors (new diagnostic-therapeutic classification of MFI).

Aims

To carry out a preliminary evaluation of this new classification of MFI in a large cohort of male infertile patients referring to the Unit of Andrology and Reproductive Medicine (University Hospital of Padua).

Results

We therefore retrospectively evaluated infertile male patients attending our Unit, collecting data in order to categorize the patients in the most appropriate category. For this preliminary analysis we excluded patients with the presence of known female problems, eventually enrolling 350 male patients with an average age of 37.5 years (average age of the partner 35.5 years). The enrolled patients were divided into the diagnostic-therapeutic categories of MFI as follows: IA (semen infection) 31/350; IB (male accessory gland inflammation) 49/350; II (congenital or acquired total obstruction): 11/350; IIIA (primary testicular disease with increased FSH): 143/350; IIIB (primary testicular disease with normal FSH): 87/350; IV (hypogonadotropic hypogonadism): 7/350; V (idiopathic semen alterations): 6/350; VI (idiopathic male infertility with normal semen analysis): 12/350. When dealing with clinically relevant varicocele, considered as a cause in itself, it was present in 4/350 patients.

Conclusions

Our preliminary data showed that it is almost always possible to find a cause for MFI; this data collection, however, is affected by the fact that it refers to a case history of patients attending a level III center, justifying the high prevalence of patients in category IIIA. This new diagnostic-therapeutic categorization of MFI is simple and rapid, also providing an indication of the possible therapeutic proposal for the male infertile patient.

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Oxidative status and hyperactivation of human spermatozoa: potential biomarkers for predicting assisted reproduction outcomes

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Introduction

Infertility is usually defined as the inability of a couple to conceive after one year of regular unprotected sexual intercourse. Approximately 17% of couples are infertile, and 50% of all cases of infertility are due to male factors^[1,2]. However, in about 15% of couples no apparent cause can be found, and the cause of infertility remain idiopathic. As a result, more couples undergoing to Assisted Reproduction Technology (ART) procedures to achieve a pregnancy, however, success rates remain low. It is known that Oxidative Status (OS) can impair sperm quality and function. Recently, our lab developed a method to evaluate sperm OS using two fluorescent probes, able to detect hydrogen peroxide and superoxide anion in viable spermatozoa: CRO and DHE^[3]. This method identifies viable oxidized spermatozoa with better characteristics. Hyperactivated motility, a sperm specific motility pattern acquired in the female genital tract, is another important parameter influencing reproductive outcomes.

Aims

The primary aim of this study was to evaluate the oxidative status and hyperactivated motility of both selected and unselected spermatozoa from male partners of infertile couples undergoing ART procedures, and to assess its correlation with ART outcomes. This study was conducted in collaboration with the Demetra ART Center in Florence (Italy), where we enrolled 179 couples undergoing ART cycles from December 2022 to June 2024. All couples were treated using the ICSI procedure. Fresh embryo transfer was possible in only 26 couples. Indications for deferred embryo transfer included: risk of ovarian hyperstimulation syndrome, elevated progesterone levels (≤ 1.5 ng/ml), and inadequate endometrial thickness on the trigger day. To eliminate potential confounding bias due to the different types of embryo transfer (fresh or frozen), we analyzed the two groups separately and found no statistically significant differences in ART outcomes between them. For this reason, we performed a cumulative statistical analysis including both fresh and deferred transfers.

Results

Preliminary results showed a significant positive correlation between the percentages of positivity for both probes, in both selected and unselected spermatozoa. Furthermore, the correlation between the percentage of spermatozoa positive for both probes, assessed before selection, positively correlated with those after selection. No significant associations were found between sperm oxidative levels and early ART outcomes such as fertilization, cleavage, blastocyst development, or embryo quality. Nevertheless, some intriguing trends were observed. Specifically, higher levels of sperm oxidation following selection were associated with embryo euploidy, biochemical pregnancy and, notably, with pregnancies that did not result in miscarriage. Additionally, although not statistically significant, higher percentages of sperm hyperactivated motility appeared to correlate with achievement of good-quality embryos, euploid embryos, and both biochemical or clinical pregnancies, supporting the idea that hyperactivated spermatozoa exhibit better functions.

Conclusion

Although preliminary, our results suggest that sperm oxidative status, assessed using both probes, is positively linked to pregnancy outcomes and negatively with miscarriage. Additionally, higher sperm hyperactivated motility appears to be associated with improved ART outcomes. These findings encourage to continue the study with the recruitment of a larger number of couples.

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Assessing the developmental competence of atypically pronucleated zygotes

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Introduction

The developmental competence of Atypically Pronucleated Zygotes (APZs) remains a debated topic in assisted reproduction. Although these zygotes are often excluded from clinical use, some studies have reported successful pregnancies resulting from them. With advancements in Artificial Intelligence (AI) and Time-Lapse Monitoring (TLM), it is now possible to analyze their developmental patterns with greater precision, providing new insights into their viability, morphokinetics and morphometrics.

Objectives

This retrospective observational study (2013-2020) analyzed 6052 inseminated oocytes, resulting in 5034 zygotes cultured in Embryoscope from 836 couples (mean maternal age: 39 years, Severe Male Factor [SMF]: 17%), to investigate the developmental competence of APZs compared to normally fertilized embryos. AI-assisted evaluation enabled the classification of different APZ types and the identification of key morphokinetic factors influencing their development. A preliminary morphometric analysis (25 embryos per group) was also conducted to explore potential correlations with the developmental competence of these embryos.

Results

Among the 6052 inseminated oocytes, AI accurately identified ProNuclei (PN) count in 89.6% of cases and corrected 1.2% of embryologists’ assessments. Among the 5034 zygotes cultured in Embryoscope, the results revealed that while some APZs reached the blastocyst stage, their success rates varied significantly based on PN count and behavior. Blastulation rates were elevated, as expected, in 2PN zygotes (54%) and reduced in APZs, with the lowest rate in 1.1PN (8%) and the highest in 2.1PN (36%). Odds ratios (ORs) were adjusted for maternal age, type of culture media (continuous vs. sequential), timing of PN appearance (tPNa), timing of PN fading (tPNf)-tPNa, and time to 2-cell stage (t2)-tPNf. APZs exhibited higher arrest rates prior to or around time to 8-cell stage (t8, 60%). tPNa occurred 2-3 hours later in APZs compared to normally fertilized zygotes. At later stages, 1PN and >3PN zygotes were the slowest to reach the expanded blastocyst stage (tEB), while 3PN zygotes reached tEB as the fastest. For each group, analysis of 25 embryos showed similar total zygote area with zona pellucida, but PN area varied across groups at both tPNa and tPNf.

Conclusions

This study highlights the need to increase the sample size for APZs and emphasizes the potential of AI in automating data collection to improve both the quality and quantity of the insights. To fully assess the clinical relevance of APZs, further research should explore additional factors such as early cleavage patterns, blastomere exclusion/extrusion, cytoplasmic strings, blastocyst morphology, chromosomal integrity, and implantation outcomes. By building on previous knowledge of APZs, including 2.1PN and 1.1PN, and leveraging AI technology, this study underscores the value of creating multimodal datasets that can predict when APZ rescue may be clinically applicable. Such data will improve communication between embryologists and clinicians, providing transparent, evidence-based information that can aid in patient counselling and facilitate informed decision-making.

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Re-expanding for success: the impact of blastocyst expansion on pregnancy rates after rapid thawing

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Introduction

Blastocyst expansion is an indicator that the blastocyst is capable of properly expressing the cellular functions necessary for implantation in the endometrium. If a blastocyst recovers quickly from thawing and reaches an adequate level of expansion, this may increase the likelihood of successful implantation. Blastocysts that re-expand and increase their degree of expansion after rapid thawing tend to have a higher implantation potential compared to those that do not re-expand or only expand partially.

Objectives

This is a cohort study addressing the pregnancy rates in relation to blastocyst expansion rate during post-thaw culture before embryo transfer. From September 2024 to December 2024, 335 embryo transfer cycles were performed at the SS PMA of Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico di Milano, involving a total of 337 thawed blastocysts with rapid warming protocol. The expansion grade was assessed at freezing and after two hours of culture in CSCN NX complete medium from thawing.

Results

A total of 337 rapid thawed blastocysts were analyzed. The median age at thawing was 37 (34–40). The freezing day was D5 (156, 46%), D6 (142, 42%), and D7 (39, 12%). Two blastocysts did not survive thawing, and 8 patients were lost to follow-up. A total of 327 blastocysts were analyzed: 246 non-re-expanded (69 pregnancies, 28%) vs 81 that re-expanded (39 pregnancies, 48%), $p=0.001$. When selecting only blastocysts with an expansion grade of 3 and 4 at thawing (according to the Istanbul Consensus ESHRE, 2011), a total of 274 were analyzed: 205 non-re-expanded (58 pregnancies, 28%) vs. 69 that re-expanded (31 pregnancies, 45%), $p=0.017$.

Conclusions

Blastocysts that showed an increase in expansion grade at rapid thawing demonstrated a higher pregnancy rate compared to those that did not re-expand. We also compared only grade 3 and 4 expanded blastocysts vs. non-re-expanded, and again, the pregnancy rate was higher in embryo transfer cycles where the blastocyst re-expanded.

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The role of cytoplasmic movements during the first 24–72 hours of human preimplantation development: an innovative AI-based assessment for predicting blastulation

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Introduction

Previous studies have highlighted the potential of AI-driven systems in optimizing human preimplantation embryo selection within IVF laboratories ^[1], demonstrating high predictive accuracy when trained on datasets derived from cultures maintained for a minimum of five days. A single study employing Time-Lapse Technology (TLT) explored the association between Cytoplasmic Movements (CMs) in early preimplantation embryos and their developmental viability ^[2]. These movements, governed by specific cytoskeletal mechanisms, represent key indicators of blastocyst formation. By utilizing computational methods, the study analysed CMs during the initial two days post-fertilization, identifying them as valuable indicators of zygote competence. Nonetheless, constraints related to limited sample size and reliance on manual data extraction underscored the necessity for large-scale, automated analyses.

Objectives

Predict embryo development into blastocyst by analysing CMs within the first 24–72 hours of culture by applying AI models to optical flow data obtained from time-lapse videos of human embryos both normally fertilized and those with an anomalous number of ProNuclei (PN).

Results

AI models (ROCKET, LSTM-FCN, ConvTran), trained on embryonic CMs data extracted through optical flow algorithms (Farneback and Lucas-Kanade), predict blastocyst development by analysing the first 24–72 hours of culture with similar performance. The analysis of the first 24 hours showed a balanced accuracy of 64% for ROCKET, 63% for LSTM-FCN, 67% for ConvTran; a sensitivity of 67% for ROCKET, 78% for LSTM-FCN, 89% for ConvTran; and a F1 score of 62% for ROCKET, 64% for LSTM-FCN, 69% for ConvTran. Extending the analysis period to the first 72 hours significantly improved performance, with a balanced accuracy of 74% for ROCKET, 76% for LSTM-FCN, 77% for ConvTran; a sensitivity of 89% for ROCKET, 85% for LSTM-FCN, 91% for ConvTran; and a F1 Score of 74% for ROCKET, 75% for LSTM-FCN, 76% for ConvTran. These results suggest that the AI models demonstrate high sensitivity, reliably identifying developmentally competent embryos while maintaining high classification performance. Clinically, this is particularly valuable as it minimizes the risk of discarding embryos that have the potential to reach the blastocyst stage, including those with an anomalous PN number, such as 1PN embryos. In fact, stratified analysis confirmed that the models perform well in predicting blastocyst formation in these abnormal embryos as well as they do in normally fertilized (2PN) embryos.

Conclusions

These preliminary results demonstrate potential for both clinical applications, such as predicting blastocyst development, and academic research, particularly in elucidating previously unrecognized cytoplasmic and cytoskeletal dynamics that influence developmental competence. These findings are promising, as the models were developed exclusively using automatically extracted data from CMs, without the incorporation of additional predictive features, manual interventions, or annotations, and utilizing only a single median focal plane.

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Comparison of artificial intelligence and traditional non-invasive methods in prioritizing euploid blastocysts for transfer: a retrospective intra-cohort analysis

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Introduction

Embryo morphology and developmental kinetics are associated with chromosomal and reproductive competence. However, traditional assessments rely on subjective interpretations and exhibit low reproducibility. Time-Lapse Technology (TLT) has facilitated continuous monitoring of preimplantation development, yet it has not significantly improved inter-observer agreement. The integration of Artificial Intelligence (AI) with TLT presents an opportunity to enhance standardization and objectivity in embryo assessment. Nevertheless, whole-chromosome testing remains the gold standard for determining embryo viability. AI-driven models aspire to provide a non-invasive prediction of blastocyst (an)euploidy, yet their clinical effectiveness must be validated within embryo cohorts to ensure their reliability and applicability in IVF settings.

Objectives

This study aimed to compare the effectiveness of AI-powered tools versus traditional non-invasive assessments in prioritizing euploid blastocysts for transfer.

Methods

A retrospective blinded analysis was conducted on 786 PGT-A cycles (maternal age: 38.9 years; study period: 2013-2020; 2184 blastocysts). Traditional static assessments were performed by three embryologists using Gardner's grading at the time of biopsy (t-biopsy). Morphodynamic evaluations incorporated time of blastocyst expansion (tEB) and embryo area at tEB (embA). AI prioritization was performed using three commercially available models. The effectiveness of each approach in ranking euploid blastocysts as top-quality was assessed in cohorts containing at least three blastocysts with different chromosomal diagnoses, including at least one euploid and one aneuploid (N=279/786, 35.5%). Coefficients of Variation (CVs = SD/mean) were calculated within cohorts containing at least three blastocysts (N=363/786, 46.2%) to evaluate intra-cohort data dispersion and its association with euploid prioritization.

Results

The intra-cohort coefficients of variation (CVs) were calculated for Gardner's grading and three AI models, revealing distinct patterns of data dispersion. Gardner's grading showed the highest intra-cohort CV (0.71 ± 0.35), while AI models 1, 2, and 3 exhibited CVs of 0.28 ± 0.14 , 0.58 ± 0.26 , and 0.56 ± 0.34 , respectively. Spearman's correlation analysis indicated moderate associations between Gardner's grading CV and AI models 1 and 2 (0.32 and 0.34, respectively), whereas model 3 displayed a weaker correlation (0.14). Notably, AI models 1 and 2 were strongly correlated (0.63) but exhibited weak associations with model 3 (0.21 and 0.22). Euploid blastocysts were prioritized in 58.4% of cases using static assessment (N=163/279), 64.2% using morphodynamic assessment (N=179/279), and in 62.4%, 68.1%, and 52.0% of cases using AI models 1, 2, and 3, respectively. Multivariate analysis revealed that a 0.1-unit increase in intra-cohort CV for Gardner's grading was significantly associated with a higher likelihood of prioritizing euploid over aneuploid blastocysts (OR: 1.1, 95% CI: 1.03-1.19, adjusted p=0.005). In contrast, no such association was observed for AI models. Additionally, aneuploid blastocysts compatible with implantation were prioritized over euploid ones in 21.1% of cases using static assessment (N=59/279), 19.4% with morphodynamic assessment (N=54/279), and in 18.6%, 17.2%, and 19.4% of cases using AI models 1, 2, and 3, respectively. A 0.1-unit increase in Gardner's grading intra-cohort CV was associated with a lower likelihood of prioritizing such aneuploid blastocysts over euploid ones (OR: 0.9, 95% CI: 0.83-0.98, adjusted p=0.010), while AI models showed no significant associations.



Comparison of artificial intelligence and traditional non-invasive methods in prioritizing euploid blastocysts for transfer: a retrospective intra-cohort analysis

Conclusions

While this study reflects real-world clinical scenarios in a large PGT-A program, it is limited by its retrospective, single-center nature and its focus on AMA patients with relatively few cycles meeting inclusion criteria. Although both traditional and AI-based grading methods correlate with euploidy, their primary goal remains embryo prioritization for transfer rather than serving as non-invasive alternatives to PGT-A. Future research should focus on improving live birth prediction rather than solely relying on non-invasive AI models as surrogates for genetic testing.

Recommended reading

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Pre-transfer progesterone evaluation in women with endometriosis or adenomyosis undergoing assisted reproductive technology

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Introduction

Endometriosis and adenomyosis, once considered incidental findings in ART patients, are now recognized as distinct conditions requiring individualized treatment strategies [1]. Progesterone is pivotal for embryo transfer success, particularly in HRT-FET cycles, where emerging evidence suggests that higher levels may improve live birth rates [2]. Recent studies demonstrate that progesterone plays a key role in regulating endometrial structure and function, particularly in the inflammatory uterine environment characteristic of these conditions. Notably, progesterone resistance may impair absorption in affected women, further compromising implantation rates [3].

Objectives

This study aims to evaluate progesterone levels in patients with endometriosis or adenomyosis versus unaffected controls to determine the need for adjustments in progesterone supplementation. Additionally, a meta-analysis incorporating recent findings by Bourdon *et al.* [1] provides a broader perspective on its clinical implications.

Results

A total of 1,927 patients were included: 327 with endometriosis, 359 with adenomyosis and 1,344 controls. Progesterone levels on the day of FET were analyzed in both medicated (MCT) and spontaneous modified (SMC) cycle protocols. A meta-analysis incorporating data from Bourdon *et al.* was performed. No significant differences in progesterone levels were observed between women with endometriosis or adenomyosis and controls, regardless of live birth or non-pregnancy outcomes, in both MCT and SMC protocols. However, among non-conceiving women, meta-analysis revealed significantly lower progesterone levels in those with adenomyosis compared to controls ($p=0.03$), with the difference being more pronounced in the diffuse adenomyosis subgroup ($p=0.02$). Additionally, within the adenomyosis group, women who achieved live births had significantly higher progesterone levels than their non-pregnant counterparts ($p=0.022$). This difference was not observed in the endometriosis group nor in the control group. Notably, in SMC subgroup, progesterone levels difference between non-conceiving women with adenomyosis and controls approached statistical significance, despite substantial progesterone production by the corpus luteum in these patients.

Conclusions

Our study demonstrates that while overall serum progesterone levels at FET do not differ significantly between women with endometriosis or adenomyosis and controls, a meta-analysis revealed that non-conceiving women with adenomyosis, especially those with diffuse adenomyosis, exhibit significantly lower levels. Moreover, among adenomyosis patients, those achieving live births had higher progesterone levels than non-pregnant counterparts, suggesting that inadequate luteal phase support may contribute to reduced reproductive success. These findings, coupled with the influence of BMI on serum progesterone, underscore the potential benefit of individualized progesterone supplementation strategies in adenomyosis patients.

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Modified natural cycle is a reasonable alternative in women over 40 years old: a retrospective analysis on 26,039 single embryo transfers

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Introduction

The natural cycle has been associated with better obstetric and neonatal outcomes compared to AC-ET, reducing the risks of pre-eclampsia, preterm birth, macrosomia, low birthweight and early pregnancy loss. However, the natural cycle is typically reserved for younger women with normal ovulatory cycles, as women over 40 years old are often considered unsuitable due to presumed ovarian dysfunction. Limited data exist on the efficacy of NC-ET in older women, despite their potential benefit demonstrated in younger women. This study investigated whether mNC-ET may be an effective alternative for women over 40 years old.

Objective

Aim of this study was to investigate whether Modified-Natural Cycle Embryo Transfer (mNC-ET) achieves a comparable Live Birth Rate (LBR) to Artificial Cycle Embryo Transfer (AC-ET) in women over 40 years old.

Material and methods

A retrospective multicentric cohort study analyzed 26,039 Single Embryo Transfers (SET) performed between January 2019 and December 2023 across 21 IVI clinics in Spain and Italy. In mNC-ET, hCG was administered at dominant follicle 13–22 mm, followed by low-dose progesterone supplementation (400 mg/day). In the AC-ET group, oral estradiol valerate (6 mg/day) was administered, followed by vaginal progesterone (800 mg/day). The primary outcome was LBR. Secondary outcomes were clinical pregnancy, ongoing pregnancy, miscarriage rates. Women aged 40–49 undergoing SET were included. Only euploid frozen ETs were considered for autologous cycles, while both fresh and frozen ETs were included for egg donor cycles. Inclusion criteria were regular menstrual cycles (21–35 days), endometrial thickness ≥ 7 mm and blastocyst-stage embryos. Exclusion criteria included double embryo transfer, severe uterine pathologies and hydrosalpinx. Statistical analyses included chi-square and Student's t-tests for group comparisons. Logistic regression adjusting for confounders was used for LBR.

Results

The study analyzed 26,039 SETs (2,694 mNC-ETs vs. 23,345 AC-ETs) in 16,579 patients (43.1 $\pm$ 2.4 y.o.). No significant differences were found in BMI, endometrial thickness, sperm source, or serum E2 and P4 levels on the day of endometrial thickness assessment. The AC-ET group included older patients (43.2 $\pm$ 2.4 vs 42.2 $\pm$ 2; $p < 0.001$), a higher proportion of egg donation cycles (84.7%, 95% CI: 84.2–85.1 vs 54.9%, 95% CI: 53–56.8; $p < 0.001$), lower proportion of PGT-A cycles (21.1% vs 51.7%; $p < 0.001$) and younger oocyte age at retrieval (27.5 $\pm$ 6.9 vs 32 $\pm$ 8.5 y.o.). No statistical differences were found in LBR between mNC and AC-ET (40.2%, 95% CI: 38.3–42.1 vs. 41%, 95% CI: 40.4–41.7, $p = 0.44$), nor in ongoing pregnancy rates (44%, 95% CI: 42.1–45.9 vs 44.3%, 95% CI: 43.7–44.9; $p = 0.78$). The mNC-ET group had lower clinical pregnancy rate (50.1%, 95% CI: 48.2–52 vs 53.9%, 95% CI: 53.25–54.53); $p < 0.001$), but also lower miscarriage rate (11.8%, 95% CI: 10.2–13.7 vs. 17.4%, 95% CI: 16.8–18.1; $p < 0.001$). Multivariate analysis adjusted for confounders (patients and oocyte age, BMI, oocyte and sperm source, endometrial thickness, serum E2 levels, PGT-A, blastocyst stage/grade) confirmed no significant LBR advantage for AC-ET over mNC-ET (OR=0.93, 95% CI 0.84–1.04; $p = 0.21$).

Conclusions

mNC-ET demonstrated non-inferior LBR compared to AC-ET in women over 40, supporting its feasibility as an endometrial preparation method in women of advanced maternal age and suggesting a shift toward individualized endometrial preparation. Further research should confirm the impact on reproductive outcomes and evaluate potential obstetric and perinatal benefits in this population.

Can modified natural cycle equalize the risks for patients over 40 years old to those for younger patients? Perinatal outcomes of 268 euploid single blastocyst cryotransfers

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Introduction

The increase in advance maternal age patients in Artificial Reproductive Techniques (ART) is becoming a challenging issue for clinicians, facing higher obstetric risks such as gestational diabetes, preeclampsia, intra-uterine growth restriction, and preterm birth. Several studies on FET in ovulatory patients have shown that mNC and the presence of a corpus luteum can reduce some obstetric risks, such as preeclampsia and preterm birth, when compared to hormonal replacement treatment protocols. Even so, few data are available regarding the obstetric risk reduction of mNC-FET in ovulatory patients up to 50y, where those risks due to advanced maternal age could potentially be reduced.

Objectives

Retrospective single-centre comparative clinical study, including 268 euploid single blastocyst ($\geq 3BB$, Gardner score) mNC-FET in regular ovulatory patients between Jan-2018 and Dec-2022: 112 patients (42%) $>40y$ ($x^- = 43,8 \pm 2,4y$, range 41-50y) and 156 patients (58%) $\leq 40y$ ($x^- = 36,5 \pm 3,0y$, range 28-40y). Both $>40y$ and $\leq 40y$ groups were homogeneous for body mass index ($22,6 \pm 4,5$ Vs $22,9 \pm 3,1$), egg donor's age ($31,0 \pm 5,9y$ Vs $32,1 \pm 5,0y$), the need for sperm donation (31,2% Vs 28,1%), and the average trophoctoderm embryo quality ($A=32,2\%$ Vs $A=33,0\%$). We studied the clinical, obstetric and perinatal outcomes of the 268 mNC-FET, comparing $>40y$ to $\leq 40y$ group for: pregnancy rate, miscarriage rate, Live Birth Rate (LBR), severe preeclampsia, preterm birth rate <37 weeks and neonatal weight. FET cancellation rate for medical reasons such as thin endometrium, spontaneous ovulation and abnormal endometrial pattern was used to adjust LBR and term LBR per started cycle. We used Chi-square and T-test to compare groups ($p < .05$).

Results

There were no statistically significant differences between $>40y$ and $\leq 40y$ when comparing FET cancellation rate (8,1% Vs 8,7%, $p=.81$) and the reason why FET was cancelled: thin endometrium (44% Vs 49%, $p=.77$), spontaneous ovulation (26% Vs 19%, $p=.57$) nor abnormal endometrial pattern (36% Vs 26%, $p=.33$). When comparing implantation and obstetric results, there were no differences between the $>40y$ and $\leq 40y$ groups in terms of pregnancy rate (56% Vs 56%, $p=.98$), miscarriage rate (15% Vs 15%, $p=.87$) and live-birth rate (45% Vs 47%, $p=.70$). Obstetric risks were non-significantly higher in $>40y$ group, almost doubling the risks seen in the $\leq 40y$ group: severe preeclampsia (1,8% Vs 0,9%, $p=.53$) and preterm birth rate <37 weeks (8,7% Vs 4,5%, $p=.36$). Neonatal weight was significantly lower in the $>40y$ group ($x^- = 3145 \pm 613g$ Vs $x^- = 3347 \pm 419g$, $p=.028$). When adjusting results per started cycle, no significant differences were found between the $>40y$ and $\leq 40y$ groups: LBR (41% Vs 43%, $p=.76$) and term LBR (38% Vs 41%, $p=.56$).

Conclusions

mNC should be encouraged for FET in ovulatory patients up to 50y because of its comparable success rates when using the same protocol in younger patients, such as LBR, even when adjusted per started cycle. However, obstetric risks when using mNC thaw cycles remain high in advanced maternal age patients.

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Maternal and perinatal risks in ART-conceived monochorionic twins: a need for tailored surveillance

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Introduction

MonoChorionic (MC) twin pregnancies are associated with a heightened risk of severe maternal, fetal, and neonatal complications. Assisted Reproductive Technology (ART) has been associated with a higher and poorly controlled incidence of MC twin pregnancies, yet the impact of ART on maternal and perinatal outcomes in this subgroup remains unclear.

Objectives

To investigate whether conception via ART is associated with increased maternal, fetal, and neonatal complications in MC twin pregnancies, and to identify areas for improved clinical management and research.

Results

A retrospective cohort study was conducted on MC twin pregnancies referred to a tertiary fetal medicine center from 2020 to 2023. A total of 100 ART-conceived MC pregnancies (77 by homologous ART and 23 by egg donation) were compared to 343 spontaneously conceived MC pregnancies. Maternal outcomes, fetal complications, and neonatal outcomes were analyzed. Logistic regression models were used to calculate Odds Ratios (OR) adjusted for maternal factors. Generalized Estimating Equations (GEE) were used to account for within-twin pair correlation. Women with ART-conceived MC pregnancies were older, more frequently nulliparous, and predominantly Caucasian ($p < 0.01$). ART-conceived MC pregnancies were associated with a significantly higher risk of pregnancy complications (aOR 3.14, 95% CI 1.76-5.60), particularly hypertensive disorders of pregnancy (aOR 3.43, 95% CI 1.23-9.53) and postpartum hemorrhage ($p < 0.05$). No significant differences concerning gestational age at delivery, birthweight, birthweight discordance and mode of delivery were found among groups. Intrauterine fetal demise was more frequent in ART pregnancies (aOR 2.09, 95% CI 1.09-4.05, $p = 0.02$), though significance was lost after adjusting for twin correlation (OR 1.77, 95% CI 0.89-3.55, $p = 0.10$). ART-conceived twins had lower rates of twin-to-twin transfusion syndrome but a significantly poorer survival after laser treatment ($p < 0.01$). Postnatal morbidity was higher in ART-conceived MC twins (aOR 1.55, 95% CI 0.91-2.63). No differences in maternal or perinatal outcomes were observed between fresh and frozen embryo transfer cycles.

Conclusions

In the largest reported cohort of ART-conceived MC twin pregnancies, we identified a significantly elevated risk of maternal, fetal, and neonatal complications. These findings raise important concerns given the global rise in ART usage. Further research is urgently needed to elucidate the underlying mechanisms—including ART-related biological factors, abnormal placental development, impaired fetoneonatal adaptation, and altered maternal cardiovascular regulation—in order to better prevent MC twinning and reduce associated risks. Tailored surveillance protocols and centralized management in specialized referral centers with expertise in ART, fetal therapy, and neonatal intensive care should be considered essential to improve outcomes in this high-risk population.

Recommended reading

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Epigenetic and clinical characterization of preeclampsia in oocyte donation pregnancies: insights into immune dysregulation and microRNA-mediated pathways

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Introduction

PreEclampsia (PE) is a severe hypertensive disorder of pregnancy that significantly impacts maternal and fetal health. It is characterized by the new onset of hypertension ($\geq 140/90$ mmHg) and proteinuria (≥ 300 mg/day) or other signs of organ system complications that develop after 20 weeks of gestation. Globally, the incidence of PE ranges from 2% to 8%, making it one of the leading causes of maternal and neonatal morbidity and mortality ^[1]. While the exact cause remains unclear, immune system dysfunction at the maternal-fetal interface is increasingly recognized as a key factor. The incidence of PE is notably higher in pregnancies following Oocyte Donation (OD), potentially due to the significant HLA mismatch between the fetus and the mother. In Natural Conception (NC) pregnancies, partial maternal-fetal HLA compatibility supports immune tolerance. However, in OD pregnancies, the fetus's genome is completely allogeneic, leading to heightened maternal immune activation and an increased risk of PE, suggesting that inadequate immune adaptation may be a key contributor to disease development ^[2]. Hormonal imbalances, such as the use of exogenous estrogen and the absence of a corpus luteum, may also contribute. Recent research has highlighted the growing role of microRNAs (miRNAs) in placental development, immune modulation, and endothelial function. miRNAs are small, non-coding RNAs that regulate gene expression at the post-transcriptional level by targeting mRNAs for degradation or translation repression. Several placenta-related miRNAs have been shown to regulate maternal immune tolerance, modulate the function of Natural Killer (NK) cells, activate Dendritic Cells (DCs), and regulate T-cell responses, thereby helping to establish the immune environment necessary for a successful pregnancy ^[3]. Disruptions in miRNA regulatory networks may lead to maternal immune activation, systemic inflammation, and impaired placentation, contributing to PE. Furthermore, circulating miRNAs have been proposed as potential biomarkers for diagnosing the progression of normal or pathological pregnancies due to their stability in maternal blood and ability to reflect placental dysfunction ^[4].

Objectives

The study aims to identify potential miRNA biomarkers associated with preeclampsia in OD and spontaneous pregnancies. This profiling may offer valuable insights into the role of miRNAs in the pathogenesis of PE, immune regulation in OD pregnancies, and the underlying disease mechanisms. Additionally, it may help facilitate the development of targeted therapeutic strategies to modulate immune responses and improve maternal-fetal outcomes.

Results

The study examined the expression of 10 miRNAs in maternal plasma, placental tissue, and umbilical cord plasma across four groups of pregnant women: Normotensive Oocyte Recipients (NOR), Preeclamptic Oocyte Recipients (POR), Normotensive Spontaneous Pregnant (NSP), and Preeclamptic Spontaneous Pregnant (PSP).

In maternal plasma, miR-17 was upregulated in PORs compared to all other groups, particularly NSPs and PSPs. miR-30 was downregulated in PORs, especially compared to NSPs and PSPs. Anti-inflammatory miRNAs miR-223 and miR-155 were upregulated in PORs compared to the other groups, with miR-223 showing higher expression compared to NSPs and PSPs, and miR-155 more expressed in PORs compared to NORs and PSPs. miR-let-7c was reduced in NSPs compared to both PSPs and PORs. No significant differences were found for other preeclampsia-associated miRNAs (miR-150, miR-140, miR-146, miR-181a, miR-210).

In placental tissue, the expression patterns were similar to those in maternal plasma, with significant differences observed in the POR group. miR-17 and miR-223 were upregulated in PORs compared to PSPs and NORs, while miR-155 was more highly expressed in PORs compared to NSPs and PSPs. miR-30 showed downregulation in PORs and NSPs compared to NORs and PSPs, and miR-let-7c was reduced in PORs compared to NORs. These trends reinforce the potential of these miRNAs as biomarkers for preeclampsia.



Epigenetic and clinical characterization of preeclampsia in oocyte donation pregnancies: insights into immune dysregulation and microRNA-mediated pathways

In umbilical cord plasma, miR-30, which was downregulated in maternal plasma of PORs, was upregulated compared to NSPs and PSPs, while miR-17 was downregulated in umbilical cord plasma of PORs compared to NSPs and PSPs. miR-223 and miR-let-7c were significantly reduced in umbilical cord plasma of PORs compared to PSPs. Additionally, miR-223, miR-155, and miR-let-7c were more highly expressed in PSPs compared to NSPs.

Conclusions

Overall, these findings suggest that miRNAs such as miR-17, miR-223, miR-155, miR-30, and Let-7c, which show differential expression across maternal plasma, placental tissue, and umbilical cord plasma, could serve as potential biomarkers for preeclampsia. The distinct miRNA expression patterns observed in the maternal and fetal compartments indicate specific dynamics of miRNA regulation during pregnancy, which could offer new insights into the early detection and monitoring of preeclampsia and other pregnancy-related complications. The upregulation of anti-inflammatory miRNAs in PORs may play a role in modulating immune responses, suggesting a protective function in preeclampsia. These miRNAs may offer valuable tools for diagnosing and monitoring pregnancy complications.

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The first IVF failure doesn't predict second attempt outcomes: the importance of a short interval

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Introduction

Failure of the first IVF cycle may lead couples to discontinue treatment before achieving a live birth, with high dropout rates significantly affecting the overall effectiveness of fertility care. Additionally, prolonged intervals between IVF attempts have been associated with lower success rates, underscoring the need for timely intervention and a multicycle strategy to optimize reproductive outcomes^[1]. However, the extent to which a patient's reproductive history—including prior failed IVF cycles^[2], embryo transfers^[3], and the absence of euploid blastocysts within embryo cohorts^[4]—predicts future success remains an area of ongoing research.

Objectives

We conducted a retrospective study analyzing 1,286 second IVF attempts performed due to DuoStim protocol (N=332), no blastocyst or only aneuploid blastocysts obtained (N=531), after ≥1 implantation-failure (N=261), ≥1 miscarriage (N=63), or ≥1 LB (N=99). We examined the association between characteristics of the first attempt – including maternal-age, cause of infertility, basal FSH and AMH, embryological outcomes, reason for cycle conclusion, retrieval-to-cycle-conclusion time, interval between attempts, and total gonadotropin dose differences – and the main outcomes of the second attempt. These outcomes included the likelihood of retrieving more Cumulus-Oocyte Complexes (COCs), the number of blastocysts obtained, the oocyte developmental competence, and the cumulative Live Birth Rate (cLBR).

Results

In their second attempt, 149 patients (11.6%) retrieved the same number of COCs, while 622 (48.4%) obtained more. A shorter interval between attempts (OR: 0.965, 95% CI: 0.952–0.978, p<0.001) and a higher total gonadotropin dose (OR: 1.46, 95% CI: 1.163–1.832, p=0.001) were associated with an increased chance of retrieving more COCs. Among patients who failed to obtain a blastocyst in their first attempt (N=399), 156 (39.1%) experienced the same outcome in their second attempt. Specifically, 326 (25.4%) obtained the same number of blastocysts, and 560 (43.5%) produced more. Maternal age (OR: 0.931, 95% CI: 0.902–0.961, p<0.001), AMH levels (OR: 1.137, 95% CI: 1.052–1.229, p=0.001), and the interval between attempts (OR: 0.969, 95% CI: 0.957–0.982, p<0.001) were significantly associated with this outcome. Oocyte developmental competence, defined as the blastocyst rate per retrieved COC, remained unchanged in 356 patients (27.7%) and improved in 521 (40.5%) during the second attempt. Maternal age (OR: 0.959, 95% CI: 0.929–0.989, p=0.009) and the time between attempts (OR: 0.977, 95% CI: 0.965–0.990, p<0.001) were associated with an increased likelihood of improvement. Among 1,222 patients who completed both cycles, the cLBR for the second attempt was 24% (N=292/1,222). This rate was independent of whether a live birth was achieved (N=120) or not (N=1,102) in the first attempt, even after adjusting for age and AMH (OR: 0.895, 95% CI: 0.516–1.553, p=0.694).

Conclusions

In second IVF attempts, the likelihood of retrieving more COCs, obtaining more blastocysts, and achieving ≥1 live birth is independent of the first attempt's outcomes. When a second IVF attempt is clinically feasible, couples should be reassured that a previous negative result does not impact on future outcomes. Moreover, shorter intervals between attempts are associated with improved outcomes, highlighting the importance of timely intervention. Therefore, adopting a multicycle counseling approach from the very first consultation is essential to avoid treatment discontinuation after the first IVF failure.

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From single child to family planning: infertility does not change the desired family size

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Introduction

Family planning is defined as a decision-making process that allows individuals and couples to regulate their fertility through the use of contraceptive methods, reproductive technologies, and educational strategies^[1,2].

Infertile couples may take a more complex path, involving medical diagnoses, treatments, and significant psychological impact. It is hypothesized that, given their reproductive history, their family planning goals may differ from those of the general population^[3].

Objectives

The aim of the present study is to assess family planning, the desire for parenthood, the approach to cryopreserved embryos and associated concerns in infertile couples undergoing an IVF cycle at Humanitas Fertility Center. We conducted a single-center survey including couples who underwent a second-level procedure (IVF/ICSI) at our center, regardless of whether it was their first cycle or a subsequent one. An anonymous questionnaire, consisting of 13 multiple-choice questions and one open-ended question, was administered to each couple in September 2024 and March 2025.

Results

A total of 142 patients participated in the study, of whom 45% were male and 54% female, with a mean age of 38.2 years. Among the couples, 89% were diagnosed with primary infertility. We found that 57% of couples desired two children, 23% desired three, 18% desired one, and 1% desired four. The mean number of desired offspring was 2.11 for females and 2.03 for males. Additionally, 64% of couples preferred achieving more than one pregnancy from a single stimulation cycle and 72.3% expect IVF to achieve the entire family plan (not just one child as soon as possible). Regarding the impact of cryopreserved material, 66.9% reported that they would not alter their family planning decisions, while 33% stated that they would. Notably, 75% of couples who had previously undergone an unsuccessful IVF cycle did not modify their family planning goals.

Conclusion

Our results indicate that the family planning practices of infertile couples are comparable to those observed in the general population. Notably, the prospect of cryopreservation offers a significant advantage, enabling couples to pursue a subsequent pregnancy and align their reproductive plans with their desired family size. Moreover, cryopreservation may serve as a pivotal factor in reshaping a couple's family planning decisions.

These findings underscore the importance of providing comprehensive family planning counseling to couples undergoing an IVF cycle.

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Reducing early discontinuation in IVF: the role of a multicycle treatment approach

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Introduction

Patient engagement is one of the key factors contributing to success in assisted reproductive technologies. Frequently, the decision-making process follows a conventional cycle-by-cycle approach, which delays treatment planning until after each failed attempt, often resulting in emotional distress and treatment discontinuation. However, growing evidence supports a multicycle approach that acknowledges the possibility of failure and highlights the benefits of planning multiple attempts in advance. This strategy aims to improve the cumulative Live Birth Rate (cLBR) and encourages patients to persist with treatment or, when appropriate, to consider oocyte donation. In this context, reducing the overall time spent in treatment—through strategies such as the use of unconventional stimulation protocols like DuoStim—plays a critical role in maintaining patient motivation and continuity throughout the IVF journey.

Objectives

This study aimed to identify factors associated with treatment discontinuation between the first and second IVF attempts. An observational study included 3542 IVF-naïve couples who underwent their first attempt between 2014–2020, with ≥ 3 years of follow-up. Median maternal age was 38 years (IQR: 35–41), median AMH 1.2 ng/mL (IQR: 0.8–2.4), and 18% had Severe Male Factor (SMF). DuoStim was performed in 328 patients. The cLBR after the first attempt was 35% (1241/3542); among those without live birth, 47% (1083/2301) discontinued treatment. Ninety-seven switched to oocyte donation after the first failure. Among second attempts, cLBR was 25% (275/1121); the conservative cLBR after two attempts was 43% (1516/3542). Another 207 patients opted for oocyte donation after a failed second attempt or DuoStim. All underwent conventional or DuoStim stimulation, ICSI, blastocyst culture, and single fresh or vitrified-warmed untested/euploid transfer. Endpoints included: cLBR per first attempt, time to complete first and two attempts, treatment discontinuation (primary outcome), time between attempts, and switch to oocyte donation. Regression analyses assessed associations between these outcomes and clinical/anamnestic variables, IVF features, and first-attempt results.

Results

Maternal age (adjusted-OR: 0.81), AMH (adjusted-OR: 1.03), and SMF (adjusted-OR: 0.75) were independently associated with cLBR. The duration of the first attempt (median: 50 days, Q1:17; Q3:120) was inversely related to maternal age (–8 days), and positively influenced by AMH (+2 days), with an extension of 26-days per blastocyst formed. When comparing cycle outcomes, those resulting in live birth had a median duration of 95 days. In comparison, cycles with no mature (MII) oocytes were significantly shorter by 126 days, and those with no transferable blastocysts were 64 days shorter. Conversely, cycles ending in implantation failure lasted 8 days longer, while those ending in miscarriage extended the duration by 56 days. Treatment discontinuation was associated with increased maternal age (adjusted OR: 1.04), lower AMH (adjusted OR: 0.98), and poor first-attempt outcomes: 46% discontinued after no transferable blastocyst, while the odds of discontinuation decreased with no MII oocyte (adjusted OR: 0.63) and increased after implantation failure (adjusted OR: 1.5). Discontinuation rates declined over time, from 57% in 2014 to 33% in 2020, during which the use of DuoStim protocols rose from 0% to 17%. Time between attempts (median: 147 days, Q1:84; Q3:248) decreased with maternal age (–6 days), increased with AMH (+1.5 days), and extended 13 days per blastocyst. Compared to no blastocyst (median: 107 days), implantation failure/miscarriage added 49 days. Time to complete two attempts (median: 193 days, Q1:85; Q3:371) decreased with maternal age (–22 days) and DuoStim use (–79 days), and increased with AMH (+4 days). Switch to oocyte donation increased with maternal age (adjusted-OR: 1.11), year of first attempt (adjusted-OR: 1.5), and DuoStim (adjusted-OR: 2.2).

Conclusions

The progressive adoption of DuoStim and oocyte donation reduced treatment discontinuation and time in treatment, supporting multicycle counseling to personalize IVF journeys and better prepare couples to deal with potential failures.



Transgenerational effect of endocrine disruptors on reproductive function

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Introduction

Endocrine Disruptors (EDs) are compounds that have the ability to affect the endocrine system by interfering with the synthesis, bioavailability, and action of hormones. Among the main EDs we can include Bisphenol A (BPA), Bisphenol S (BPS) and Perfluoroalkyl compounds (PFAS) [1-3].

Aim

In this PRIN study (Grant 20203AMKTW_002) we evaluated the possible transgenerational effects of BPA, BPS and PFOS on testicular function in male F1 mice born from mothers treated in pregnancy and during lactation with subtoxic levels within the recognized tolerability levels for these three substances: BPA (4 ug/kg b.w., N=12); BPS (4 ug/kg b.w., N=14) and PFOS (3 ug/kg b.w., N = 15).

Results

Mice were sacrificed at sexual maturity (P = 90 days), and subsequently blood was collected, testes were stocked at - 80°C, and spermatozoa were retrieved from their respective epididymis to analyze the effects of different EDs by assessment of sperm count and motility, quantification of plasma hormones by ELISA method, and analysis of protein expression by western blotting technique. Compared with the unexposed controls ($49.14 \pm 19.87 \times 10^6$ spermatozoa), the group exposed to PFOS ($12.19 \pm 3.17 \times 10^6$ spermatozoa), or BPA ($10.12 \pm 4.8 \times 10^6$ spermatozoa) or BPS ($8.84 \pm 2.0 \times 10^6$ spermatozoa) showed reduced sperm concentration ($P < 0.05$). In parallel, the percentage of sperm with progressive motility dropped dramatically (-90% on average) in all three ED-exposed groups compared with the control group. Quantification of hormones in plasma showed decreased levels of testosterone and INSL3 in the three groups of exposed mice compared with controls (all $p < 0.05$), while FSH and LH levels were increased in exposed mice compared with control (all $p < 0.05$). Protein expression analysis in the testis showed increased FSH receptor in all three ED-exposed groups compared with the control. Finally, bioaccumulation of BPA, BPS and PFOS was evaluated by LC-MS, showing that all three EDs were concentrated at the testicular level (BPA= 237.07 ± 119.02 ng/g; BPS= 306.45 ± 140.09 ng/g; PFOS= 60.75 ± 35.66 ng/g) compared to plasma (BPA= 216.44 ± 137.24 ng/mL; BPS= 120.81 ± 62.98 ng/mL; PFOS= 7.76 ± 3.78 ng/mL), all $p < 0.05$ compared with control.

Conclusions

This study demonstrates for the first time a transgenerational effect of EDs at subtoxic dosages on testicular function, with direct damage on spermatogenesis and Leydig cell function, with intratesticular accumulation of these molecules

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