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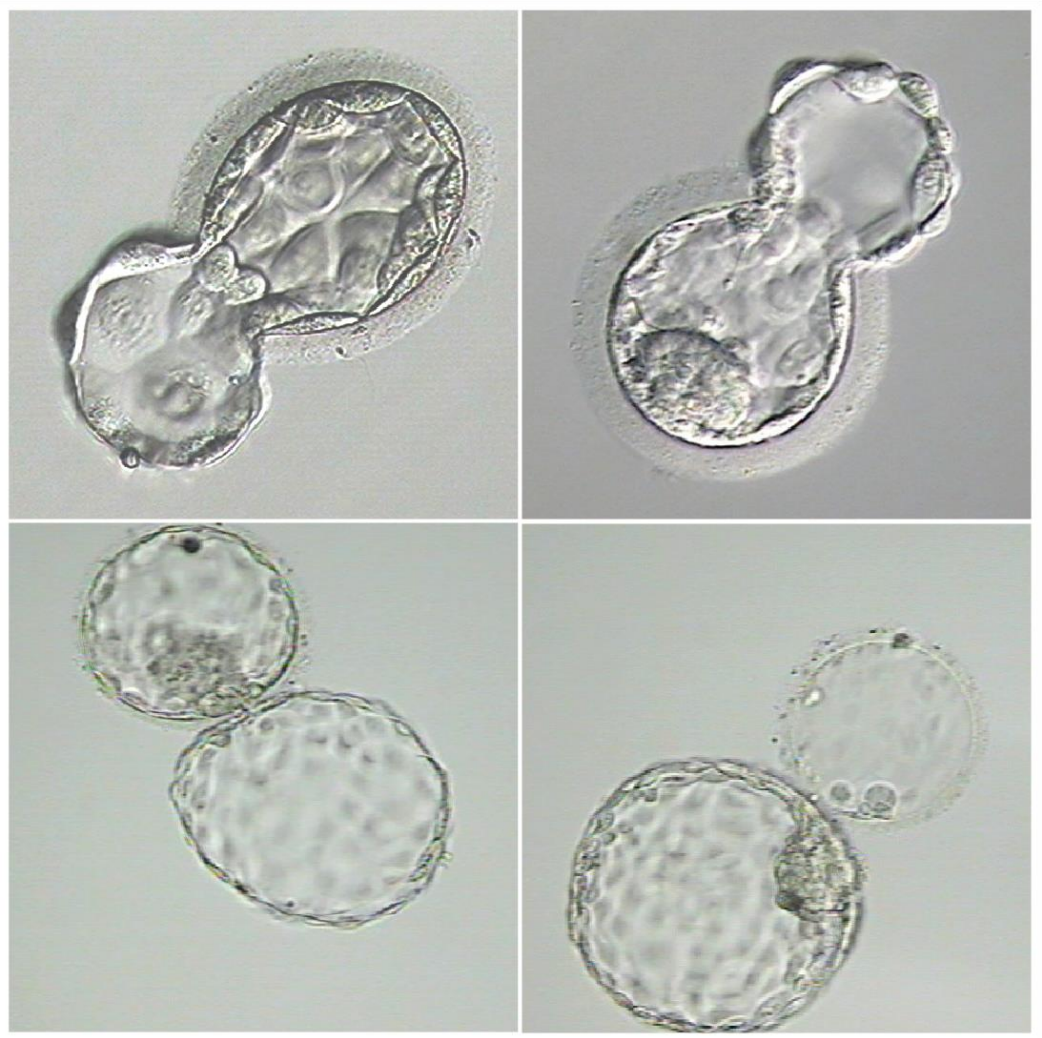

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Table of Contents

Editorial

Editorial ____ 1

Opinion

Evolution of Reproductive Medicine ____ 3

Reviews

Relationship between Polycystic Ovary Syndrome and Metabolic Syndrome ____ 5

Research Articles

Assessing endometrial receptivity after recurrent implantation failure in euploid embryo transfer: a retrospective study in private clinic ____ 15

Surgical sperm extraction versus semen centrifugation: Method of spermatozoa recovery does not correlate with euploidy rates in patients with cryptozoospermia ____ 22

Embryo Transfer is the last Frontier for Deep Machine Learning & Artificial Intelligence in Medically Assisted Reproduction (MAR) ____ 28

Case Reports

Importance of pre-test genetic counselling in couples undergoing assisted reproductive techniques (ART) and preimplantation genetic testing (PGT) ____ 39

Beckwith-Wiedemann Syndrome in a patient with full-term pregnancy, case report ____ 43

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Editorial

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Dear Readers,

It is our pleasure to welcome you to the second issue of The Journal of Reproduction (TJOR). We are delighted with the positive response received for our first issue, as over 10,000 readers have engaged with the insightful articles published by researchers from diverse geographic locations.

Our hope is that this issue will continue to be well-received and inspire clinicians, biologists, psychologists, and other professionals in the reproductive health field to share their own experiences, thereby allowing colleagues from around the globe to benefit from their expertise.

We have a few announcements that we would like to share with you. Firstly, in addition to our regular quarterly issues, we will be publishing special issues focused on specific topics related to reproduction. These will cover a range of topics such as surrogate motherhood, the use of artificial intelligence (AI) in assisted reproduction, and novel approaches to treating infertility such as mitochondria transfer, spindle transfer and others. We have already reached out to top leaders in each field from different parts of the world to contribute articles. Our first special issue is planned to be published on September 1st, 2023.

Secondly, we are pleased to announce that TJOR will continue to be completely free to register, read, and publish manuscripts. To support this, we will be introducing promotional pieces from commercial companies. In this issue, our friends from Ovogene are the first to participate. We anticipate that in the future, other companies related to the field of reproduction, including pharmaceuticals, genetics laboratories, and producers of IVF laboratory instruments, will be interested in showcasing their products and work in our journal.

Lastly, we are proud to announce that TJOR is making significant progress towards achieving international certifications for inclusion in PubMed for full references approval. We have completed a lengthy process involving administrative, academic, and legal steps to obtain our certification with ISSN (International Standard Serial Number) and DOI (Digital Object Identifier) generated by Crossref. The benefits of having an ISSN include international publicity and recognition of serials by automatic inclusion in the International Serials Directory Database. An ISSN is an 8-digit code used to identify newspapers, journals, magazines, and periodicals of all kinds, both print and electronic. A DOI is a unique string of numbers, letters, and symbols used to identify an article or document and provide it with a permanent web address (URL). Including a DOI in your citation helps readers easily locate a document. These certifications will facilitate our presence in PubMed Central and publisher websites in the near future.

We look forward to receiving your comments on our second regular issue and hope that you find the articles

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informative and engaging. Please accept our warmest regards.

Sincerely,

Asch-Schuff Ricardo Héctor

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Opinion

Marlene Lizbeth Zamora Ramírez[2], 0000-0003-2871-3646.

Evolution of Reproductive Medicine

Reproductive medicine (RM) has evolved significantly over the past few decades, and we have moved "overnight".

The first fertility treatments such as artificial insemination date back to the 1950s. In the 1970s in vitro fertilization (IVF) was developed.

The advances of RM have been due to the great technological development of pharmacology, imaging, reproductive surgery, molecular biology, andrology and reproductive genetics, as well as assisted reproduction laboratories (ARL): culture media, insemination techniques, embryo biopsy taking, vitrification-devitrification, non-invasive monitoring of embryos, the creation of preimplantation genetic analysis (PGT-A and M, Sr) which has improved the effectiveness of assisted reproduction techniques (ART).

New massive sequencing techniques have changed our perspective and focus. Today it is possible not only to solve fertility problems, the transmission of diseases of genetic origin can be prevented, which a few years ago could not be detected, it is even possible to replace altered genetic material, although these techniques are still in research phases, we will have to see their effectiveness.

The lines of research in RM today focus on the creation of techniques for egg development, disease prevention, non-invasive preimplantation genetic testing, improving the implantation potential of the endometrial and embryonic component.

The future of RM will be the implementation of artificial intelligence (AI) in ARL, as well as their robotization. All with the aim of increasing the efficiency and effectiveness of ART, to solve not only male and female fertility problems, but also to prevent disease.

The demands as a society have also changed, with the professionalization of women, the postponement of fertility by women, couples, and the evolution of sexual diversity.

Today women and men have the option to preserve fertility; freezing eggs, sperm and embryos, whether the cause is due to a disease such as cancer, or for personal reasons, or professional circumstances, are forced to postpone motherhood.

The important thing is to ask where the future prospects are going, where is the limit of what is technically possible and what is ethically acceptable? And do not lose the objective: optimize the results and ensure the birth of a healthy baby, and not the idea of a custom-made baby.

The journal wants to motivate the dissemination of research in the world on reproductive medicine, which surely in 10 years will not be the same as we are living, but will have the same objective everyone can have a baby and form a family both women, men, couples with fertility problems, as the SD and form the different types of family either single-parent two-parent,

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homoparental, as marked by the World Health Organization, and there are no political, social, cultural, religious, geographical, or economic limitations.

Marlene Lizbeth Zamora Ramírez. MD. G&O. Reproductive Medicine

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Relationship between Polycystic Ovary Syndrome and Metabolic Syndrome.

Héctor Iván Saldívar Cerón

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a heterogeneous, complex, and widely misunderstood endocrine disorder that affects intermediate metabolism, the cardiovascular and reproductive systems, and has social and psychological consequences. The prevalence and incidence of PCOS is high among women with Metabolic Syndrome (MetS). In Mexico, the hyperandrogenic subtype is the most prevalent of the characterized subtypes and is characterized by hyperandrogenism, insulin resistance, and ovulatory dysfunction. Central obesity plays a role in the development of the hyperandrogenic subtype as adipositopathy can cause a syndrome of insulin resistance and androgen excess, both of which contribute to cardiovascular comorbidities. These two syndromes share similar pathophysiological mechanisms, suggesting that PCOS could be a complication of MetS or vice versa. The purpose of this article is to explore the relationship between MetS and PCOS, with a focus on pathogenesis, infertility, microbiota, comorbidities, and treatment.

KEYWORDS: Metabolic syndrome, Polycystic Ovary Syndrome, fertility, insulin resistance, microbiome.

1 INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in reproductive-aged women, characterized by polycystic ovaries, hyperandrogenism, anovulation, menstrual irregularities, and weight gain. It is estimated to affect approximately 7% of reproductive-aged women and is one of the most common causes of infertility.[1]

Metabolic syndrome (MetS) is a group of medical disorders, including central obesity, high blood pressure, insulin resistance, hyperglycemia, and hyperlipidemia, that are associated with an increased risk of cardiovascular disease and type 2 diabetes. Women with PCOS have been shown to have a higher prevalence of MetS compared to women without PCOS. In addition to its association with MetS, PCOS is also associated with a higher incidence of infertility. Anovulation and hyperandrogenism are key factors in infertility associated with PCOS. Insulin resistance and weight gain may also contribute to infertility in women with PCOS, as these factors can affect ovulation and ovarian function. PCOS is the most common endocrine disorder that leads to infertility in women. Despite its

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name, the "cysts" seen in the ovaries are accumulations of follicles in varying stages of maturation and atresia, which only represent one aspect of this complex syndrome.[2-4]

Attempts have been made to rename this condition, but international expert societies and academies in the field have not yet agreed on a more accurate name, due to the different phenotypes that the disease can take. For example, the Rotterdam definition requires 2 of the following 3 criteria for a diagnosis of PCOS:

1) hyperandrogenism,
2) ovulatory dysfunction, and
3) oligoovulation and polycystic ovarian morphology (PCOM).

The Androgen Excess and PCOS Society (AE-PCOS), on the other hand, requires the presence of hyperandrogenism with ovarian dysfunction and PCOM. The National Institute of Child Health and Human Development, meanwhile, does not consider PCOM, but mandates hyperandrogenism and ovarian dysfunction.[5-7]

Given the heterogeneity of PCOS in terms of both pathophysiology and severity, it's possible that we are dealing with multiple entities that are being grouped under one name. Some women have hyperandrogenism without insulin resistance and a normal weight, while others have clear insulin resistance, metabolic and reproductive dysfunction, are overweight/obese, and have hyperandrogenism and PCOM. There is currently no solid basis for categorizing each group. However, in Mexico, we have observed that the prevailing PCOS phenotype is linked to cardiometabolic disorders, insulin resistance, hyperandrogenism, and PCOM, which is associated with the increase in the incidence and prevalence of MetS in our country.[8-9]

MetS is defined by central obesity, high blood pressure, insulin resistance, and dyslipidemia, and its long-term complications include the development of type 2 diabetes, cardiovascular disease (coronary heart disease, stroke), cancer, obstructive sleep apnea, psychological/psychiatric problems, infertility, and reproductive system disorders. It's important to note that the pathophysiology and long-term consequences of hyperandrogenic PCOS are like MetS, as if they were the same entity and PCOS was just another complication (Figure 1). Some authors argue that PCOS is the cause of the development of MetS, but evidence shows that hyperandrogenism is not the cause of insulin resistance, as the pharmacological suppression of androgens does not improve insulin resistance. It's not yet clear if PCOS is a manifestation of MetS or vice versa.[10-11] This review examines the current relationship between PCOS and MetS, highlighting their similarities and offering a critical perspective for medical professionals when dealing with patients with both syndromes.

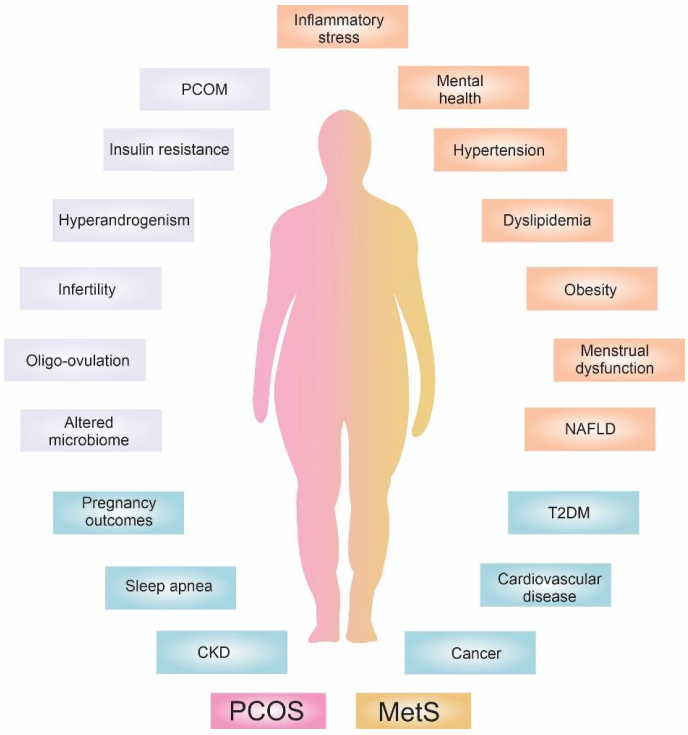

Figure 1. Interrelationship between PCOS and MetS. The interrelation between PCOS and MetS is a result of a vicious circle where excessive visceral adipose tissue increases androgen production, which in turn can trigger PCOS. However, women with PCOS also have a higher risk of developing obesity due to insulin resistance. As a result, MetS and PCOS feed each other. This potentiates the presence of short-term comorbidities such as mental health problems, menstrual disorders, infertility, acanthosis nigricans, etc., and in the long term: type 2 diabetes (T2DM), chronic kidney disease (CKD), increased risk of cancer, cardiovascular diseases, obstructive sleep apnea, non-alcoholic fatty liver disease and liver cirrhosis, leading to patient death. It is important to identify and refer both syndromes to address these comorbidities and prevent any long-term health problems.

2 Pathogenesis

PCOS is a heterogeneous syndrome characterized by an excess of androgens (hirsutism and/or hyperandrogenemia) and ovarian dysfunction (oligoovulation and polycystic ovarian morphology). The etiology remains unknown, but there is evidence of it being a multigenic and epigenetic disease with influences from the external environment, especially lifestyle factors. Different phenotypes of PCOS have been described:

1) hyperandrogenism with ovulatory dysfunction,
2) hyperandrogenism with PCOM,
3) oligo-ovulation with PCOM,
4) hyperandrogenism, oligo-ovulation, and PCOM.[8, 12]

In terms of pathophysiology, insulin resistance and hyperinsulinemia are the most important mechanisms in the development of PCOS. This is because in a state of overweight/obesity, adipositopathy promotes a state of peripheral insulin resistance and compensatory hyperinsulinemia that contributes to excessive androgen production. Furthermore, insulin acts as a gonadotropin in the ovary, facilitating the secretion of suprarenal androgens and modulating the pulsatile secretion of LH. In turn, androgens contribute to dysfunction of the adipose organ, generating a vicious circle of excess androgens that favors the deposit of abdominal fat tissue and visceral adiposity by inducing insulin resistance and compensatory hyperinsulinemia, which further facilitates androgen secretion by the ovaries and suprarenal glands. From this perspective, the most important factor is obesity, abdominal adiposity, and insulin resistance, elements that are fundamental to the development of MetS. Because apparently the heterogeneity and severity of PCOS is due to a possible MetS, genetic and epigenetic factors would play an important role in the development of different phenotypes and subphenotypes reported. When the environmental factors related to the development of PCOS and MetS are analyzed, we observe that they share the same etiopathogenesis. Briefly, intrauterine growth delay and insulin resistance induce a thrifty phenotype that leads to overweight problems in childhood, which can progress to obesity, hypertension, insulin resistance, dyslipidemia, and PCOS, as if PCOS were a lost milestone in the natural history of MetS. Many answers to the multiple questions of the origin and complexity of PCOS are still outstanding.[13-16]

3 Infertility

PCOS is the most common causa of infertility in women. This is because PCOS can cause irregular menstrual cycles and the formation of cysts on the ovaries, which can prevent ovulation from occurring. As a result, women with PCOS may have trouble getting pregnant without medical intervention. Treatment options for infertility in women with PCOS may include medications to induce ovulation, such as clomiphene citrate or letrozole, or assisted reproductive technologies like in vitro fertilization (IVF). Maintaining a healthy lifestyle, including a balanced diet and regular exercise, can also help improve fertility in women with PCOS.

Due to a poor understanding of the mechanisms involved, there are limited options in the specific etiological treatment for infertility, however, once pregnancy is achieved, PCOS predisposes to the development of gestational diabetes, spontaneous abortion, hypertension in pregnancy, preeclampsia, pregnancy preterm which increases the risk of neonatal death, complications shared in women with MetS in the fertile stage[17].

3.1 Miscarriage

Although the main causes of spontaneous abortion are associated with chromosomal abnormalities, it is also linked to metabolic disorders such as obesity, MetS, diabetes mellitus, and PCOS Several studies have found that PCOS is not a direct cause of spontaneous abortion, as the rates of spontaneous abortion among women with PCOS were similar to those among women without PCOS. However, when PCOS is present in women with high Body Mass Index (BMI), a strong association has been observed, suggesting that BMI may be a confounding variable, as obesity is a known cause of spontaneous abortion. Given that both PCOS and MetS are prevalent in Mexico, it is possible that the presence of both conditions could contribute to the high rate of spontaneous abortions among women with both syndromes. The mechanism by which PCOS may lead to spontaneous abortion is not yet clear, but potential mechanisms include insulin resistance, hyperhomocysteinemia, hyperandrogenemia, PAI1, vitamin D binding protein, and MetS. Further studies are needed to fully understand the association between these syndromes and spontaneous abortion.[18-22]

3.2 Gestational Diabetes Mellitus.

Gestational Diabetes Mellitus (GDM) is characterized by a state of glucose intolerance that develops in the second trimester of pregnancy and is linked to insulin resistance, hyperglycemia, oxidative stress, and chronic inflammation. The connection between MetS and GDM is widely acknowledged, and it is crucial to identify patients who may be at risk for developing GDM. However, the relationship between GDM and PCOS is unclear. Several studies have not

yet confirmed the extent to which the relationship between GDM and PCOS is independent of MetS. Some authors suggest that PCOS is not a standalone risk factor for the development of GDM, as pregnancies that occur with PCOS often come with additional risk factors, such as high Body Mass Index (BMI). To date, there is no study that clarifies the relationship between GDM and PCOS without the influence of obesity as a confounding factor. This could be due to the fact that the attempt to separate PCOS from MetS is misguided, as PCOS could actually be considered a bi-syndrome.[23-26]

3.3 Preeclampsia

Pregnancy-induced hypertension is defined as a systolic blood pressure >140 mmHg and/or a diastolic blood pressure >90 mmHg on two separate occasions with at least 2 hours difference, that occurred either before pregnancy or after 20 weeks of gestation. MetS is a risk factor for preeclampsia, and similarly to gestational diabetes mellitus (GDM), there is a confounding factor between obesity and PCOS and preeclampsia. Several meta-analyses have tried to clarify the role of PCOS in the development of pregnancy-induced hypertension and although the results are polarized, it seems that there are a number of mechanisms that potentially support the association between PCOS and preeclampsia, many of which are shared with MetS. Some of these mechanisms include insulin resistance and hyperinsulinemia, hyperhomocysteinemia, restriction of coenzyme A, peripheral vascular resistance, hyperlipidemia, chronic inflammation, among others. Again, more clinical studies are needed to decipher the interaction between MetS, PCOS, and preeclampsia.[27-30]

3.4 Preterm birth

PCOS has been associated with preterm birth (Preterm birth is defined as birth before 37 weeks of gestation) in several studies, although the exact mechanisms linking the two are not well understood. The association between PCOS and preterm birth is partly due to the history of assisted reproduction, as previously it increased the risk of multiple births and hypertensive diseases, which are associated with the development of preterm birth. With regards to the proposed mechanisms, these include obesity, MetS, nulliparity, hyper-estrogenemia, hyperinsulinemia, GDM, meta-inflammation, and hyperandrogenism. Although there appears to be an association between PCOS and preterm birth, the reported risk factors are mainly associated with MetS, which highlights the need for further research to fully understand the link between PCOS and preterm birth.[31-33]

4 Microbiome

The intestinal microbiome is a collection of microorganisms that reside the gastrointestinal tract, with the majority belonging to four phyla: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria and others. These microorganisms maintain a stable relationship with the intestinal epithelium and form a stable community that performs complementary functions and interacts with the host.

Changes in the gut microbiome have been linked to the development of metabolic disorders such as type 2 diabetes and obesity. There is evidence that the gut microbiome differs between people with metabolic disorders and those who are healthy. Intestinal dysbiosis is associated with chronic inflammation, insulin resistance, excessive androgen secretion, dyslipidemia, and obesity, conditions that are common in PCOS and MetS.[34]

In a recent study, Saturina et al. observed that it was possible to distinguish significant differences in the microbiome of PCOS patients and those without PCOS. These findings are controversial compared to previous reports that found no significant differences. This discrepancy may be due to the fact that Saturina et al. classified PCOS patients based on phenotypes. On the other hand, Lull et al. reported a negative correlation between the Shannon index and body mass index, fasting insulin, and free androgen index, and a positive correlation with sex-hormone-binding globulin, Matsuda index, and disposition index. While some studies have not found clear changes in the microbiome related to hyperandrogenism, a pathological metabolic state may reveal that changes in the microbiome are influenced by MetS rather than hyperandrogenism. There is ample evidence to support that MetS patients have a distinct gut microbiome compared to healthy individuals. The gut microbiota affects women with PCOS by promoting insulin resistance and increasing gut permeability, leading to chronic inflammation and subsequent excessive androgen secretion. Further research is needed to determine if there is a difference in the microbiome of Mexican women with PCOS and PCOS/MetS compared to healthy women.[35-37]

5 Comorbidities

PCOS and MetS share several comorbidities, including:

1) Obesity: Both PCOS and MetS are often linked to obesity, which can exacerbate the symptoms and comorbidities associated with the conditions,

2) Insulin resistance: Women with PCOS are at an increased risk of insulin resistance, while MetS is characterized by elevated insulin levels,
3) Cardiovascular disease: PCOS and MetS both increase the risk of heart disease, high blood pressure, and stroke,
4) Type 2 diabetes: PCOS and MetS both increase the risk of developing type 2 diabetes,
5) Dyslipidemia: Both PCOS and MetS are associated with alterations in cholesterol and triglyceride levels, which can increase the risk of cardiovascular disease,
6) Sleep apnea: PCOS and MetS both increase the risk of obstructive sleep apnea,
7) Mental health issues: Women with PCOS and MetS may experience depression, anxiety, and other mood disorders.

It's important for women with PCOS and MetS to be evaluated and monitored regularly by a healthcare provider to address these comorbidities and prevent any long-term health issues. For a more detailed review, consult the review article by Emily W. Gilbert et. al.[38]

5.1 PCOS and COVID-19

PCOS and SARS-CoV-2 (COVID-19) infection are two different medical conditions that affect different bodily systems. However, there is some evidence that women with PCOS may have an increased risk of serious complications from COVID-19. The relationship between PCOS and COVID-19 is discussed, with evidence suggesting that women with PCOS have a significant risk of hospital complications and mortality during the COVID-19 pandemic due to factors such as insulin resistance, central obesity, and hyperandrogenemia. Additionally, epidemiological data suggests that women with PCOS have a 28% to 50% higher risk of contracting SARS-CoV-2 and that COVID-19 is associated with higher rates of hospitalization, morbidity, and mortality in these women. It has also been seen that obesity worsens the COVID-19 situation due to the increase in proinflammatory cytokines, and that insulin resistance and hyperinsulinemia, which are common in PCOS, explain the association between PCOS and a more prevalent infection with SARS-CoV-2. PCOS is a common endocrine disorder that affects approximately 5-10% of women of reproductive age. It is characterized by irregular ovulation, increased production of androgen hormones, and the formation of ovarian cysts. Many women with PCOS have obesity, hyperinsulinemia, and insulin resistance, which makes them more prone to developing metabolic diseases such as type 2 diabetes and cardiovascular disease. On the other hand, COVID-19 is an infectious disease caused by the SARS-CoV-2 virus that spreads through contact with respiratory droplets from an infected person. COVID-19 can cause mild to severe symptoms, including fever, cough, difficulty breathing, and in severe cases, respiratory failure and death. PCOS is a different clinical entity from the MetS, but both can be related. Many women with PCOS have risk factors for the MetS, such as obesity, insulin resistance, and hyperinsulinemia. However, there are also women with the MetS who do not have PCOS. Therefore, it can be said that PCOS and the MetS are two distinct, but related conditions.

In recent studies, it has been demonstrated that women with PCOS have a higher risk of developing serious complications from COVID-19, including hospitalization and the need for mechanical ventilation. This may be due to a combination of factors such as obesity, insulin resistance, and hyperandrogenemia, which are known to increase the risk of serious COVID-19 illnesses. Obesity can trigger PCOS because excess body fat can increase the production of androgen hormones, which in turn can trigger PCOS. However, women with PCOS also have an increased risk of developing obesity due to insulin resistance and other health problems related to PCOS. Therefore, it can be a vicious circle, where obesity and PCOS feed into each other. Insulin acts as a gonadotropin in women with PCOS due to insulin resistance that characterizes the disease. Insulin resistance causes insulin levels to increase in the body, which in turn stimulates the production of androgens in the ovaries. These excessive androgens can trigger the growth of ovarian cysts, which are a distinctive feature of PCOS. Furthermore, high insulin levels can also interfere with normal ovulation, which can contribute to the development of PCOS. It has been seen that women with PCOS have a higher risk of contracting COVID-19 due to the presence of comorbidities associated with PCOS, such as insulin resistance, central obesity and hyperandrogenemia. These factors, along with age and other metabolic diseases, can increase vulnerability to infection with the SARS-CoV-2 virus. In addition, it has been demonstrated that obesity exacerbates the course of COVID-19 disease, which explains the association between PCOS and a higher prevalence of SARS-CoV-2 infection. In conclusion, although PCOS and COVID-19 are two different medical conditions, there is some evidence that women with PCOS may have a higher risk of serious complications from COVID-19.[39-41]

6 Recent advances in emerging PCOS therapies.

The current treatment for PCOS with metabolic features depends on the patient's goals. For women not seeking fertility assistance, combined oral contraceptive pills (OCs) are commonly prescribed to

treat menstrual irregularities and physical manifestations of hyperandrogenism such as hirsutism, acne, and alopecia. These pills contain both estrogen and progestin and reduce gonadotropin release and androgen production. Anti-androgen medications may also be used if needed. For metabolic symptoms, weight loss and dietary changes, as well as oral insulin sensitizers like metformin, are recommended. For women seeking fertility treatment, weight loss and ovulation induction agents such as letrozole or clomiphene citrate with metformin may be advised, but these treatments increase the risk of ovarian hyperstimulation syndrome.[42]

6.1 Novel treatments for PCOS.

Although current treatment strategies for the endocrine, metabolic, and reproductive aspects of PCOS are effective, there is still a need for improvement. Firstly, many current therapies are associated with adverse side effects, such as weight gain associated with oral contraceptive use, gastrointestinal problems related to metformin and an increased risk of ovarian hyperstimulation syndrome (OHSS) in fertility treatments. Secondly, hormonal treatments are not always appropriate, for example in individuals where estrogen therapy is contraindicated, such as breast cancer, venous thrombosis, and stroke. Fortunately, there are several novel treatments for the management of PCOS that are demonstrating their potential in preclinical animal models and early clinical trials.[43-45]

6.2 New treatments targeting excess androgens.

Hyperandrogenism contributes to the central pathogenesis of PCOS and underlies many of the obvious and problematic symptoms for patients with PCOS. According to this, animal models replicating the metabolic and reproductive features of PCOS are often generated by prenatal or peripubertal exposure to excess androgens. Therefore, therapeutic reduction of androgens or androgen receptor (AR) blockade are important strategies in the treatment of PCOS. Early antiandrogenic intervention may also be critical for improving fertility outcomes. A population-based retrospective study in Sweden found that women with PCOS who had early antiandrogenic intervention (before the age of 18) had improved fertility rates compared to those with later interventions. Furthermore, studies in mice indicate that excess androgens can have long-term impacts on follicle and oocyte quality that may continue to affect fertility even after restoring hyperandrogenism. Direct antagonists of androgen receptors may interact with GABA-A receptors in the brain, increasing the risk of seizures.[46-48]

6.3 New treatments targeting neuroendocrine dysfunction.

Clinical targeting of neuroendocrine dysfunction involves the modulation of GnRH and its inputs within the GnRH neuronal network to treat conditions such as PCOS GnRH antagonists, such as cetrorelix, have shown promise in treating PCOS in animal models. Modulation of kisspeptin through a kisspeptin receptor agonist, MVT-602, has been investigated as a therapeutic strategy in a small clinical trial, with promising results. Modulation of neurokinin B through its antagonist fezolinetant has also been studied in clinical trials and has shown reduction in LH and testosterone levels in women with PCOS. Another avenue of therapy is to target the dynorphin receptor kappa opioid to enhance dynorphin-mediated inhibition of kisspeptin secretion. Overall, the modulation of GnRH inputs within the GnRH neuronal network offers promising avenues for the treatment of PCOS.[49-51]

6.4 New treatments targeting insulin resistance.

Treatments targeting insulin resistance in PCOS aim to reduce hyperinsulinemia and insulin resistance that commonly occur in both obese and non-obese women with PCOS. This reduction helps to reduce hyperandrogenism, which exacerbates ovarian androgen production and reduces SHBG (Sex Hormone Binding Globulin), leading to increased testosterone levels. Due to limitations and adverse effects of metformin, alternative insulin sensitizing agents are being researched, including humanin analogues, sodium glucose co-transporter inhibitors, and incretin mimetics. Humanin is a peptide with protective effects under stress conditions in various cell types, including gonadal cells. Decreased humanin expression has been found in PCOS patients with insulin resistance. Studies in DHEA-induced PCOS rats showed humanin supplementation improved fasting glucose and insulin levels and decreased body weight gain. Sodium glucose co-transporter inhibitors, such as SGLT2 inhibitors, are anti-diabetic drugs used in treating type 2 diabetes. They improve insulin sensitivity and blood glucose levels and can cause weight loss. Clinical trials comparing SGLT2 inhibitors to metformin in overweight/obese PCOS patients showed benefits including decreased body weight, serum DHEAS, and fewer adverse effects. A dual SGLT1/2 inhibitor (LIK066) showed promising results in reducing insulin and androgen levels in a 2-week Phase 2 trial in PCOS women. Incretin mimetics, such as GLP-1 receptor (GLP-1R) agonists, have shown promising results in treating PCOS. Clinical trials found that GLP-1R agonists, such as Liraglutide and Semaglutide, reduced free androgens and body weight, and improved insulin sensitivity compared to placebo or metformin. There is some evidence that GLP-1R analogues modulate GnRH release and LH

surge in rats. Although the results of these treatments are promising, further studies with larger sample sizes and direct comparisons are needed to fully establish their efficacy and safety for use in treating PCOS.[52-54]

6.5 PCOS and clinical trials.

Although there are multiple treatments aimed at treating the symptoms of PCOS, there is no specific treatment approved by either the FDA or the European Medicines Agency. Currently, there are 749 records of clinical trials on ClinicalTrials.gov addressing PCOS and 223 addressing the relationship between PCOS and MetS, however, the number of studies involving a treatment is limited. In the pharmaceutical industry, there are only 12 studies addressing PCOS and obesity, including low-dose contraceptive treatments, exenatide, dapagliflozin, exenatide plus dapagliflozin, dapagliflozin plus metformin, liraglutide, D-chiro-inositol, metformin, and orlistat, and phentermine with topiramate. And only 25 records by NIH that include low-carbohydrate dietary intervention, flutamide, oral contraceptives, stress management-focused psychological treatment, iDPP4, adrenergic receptor agonists, leuprolide acetate, and spironolactone. Therefore, from a pharmacological treatment perspective, PCOS may currently be considered the most prevalent orphan disorder among adolescent and adult women.

7 Conclusion

In conclusion, PCOS is a common endocrine disorder in reproductive-aged women that is characterized by polycystic ovaries, hyperandrogenism, anovulation, menstrual irregularities, and weight gain. PCOS is also associated with a higher prevalence of MetS, which is defined by central obesity, high blood pressure, insulin resistance, and dyslipidemia. The exact pathogenesis of PCOS remains unknown, but there is evidence that it is a multigenic and epigenetic disease that is influenced by lifestyle factors, particularly obesity and insulin resistance. PCOS and MetS have similarities in terms of pathophysiology and long-term consequences, and it's not yet clear if PCOS is a manifestation of MetS or vice versa. Further research is needed to better understand the relationship between PCOS and MetS.

This syndrome is one of the most poorly understood by both patients and physicians. It has been wrongly perceived as a temporary condition with limited impacts on fertility, but the lack of understanding about its long-term consequences has led to deaths in our country. One reason for this misunderstanding may be due to the incorrect terminology; Polycystic Ovary Syndrome (Hyperandrogenic) should more accurately be referred to as a MetS affecting the female reproductive system and its associated consequences. By adopting this proper terminology, we can better address and prevent cardiovascular and metabolic complications.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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Assessing endometrial receptivity after recurrent implantation failure in euploid embryo transfer: a retrospective study in private clinic

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ABSTRACT

Objective:

To analyze whether endometrial receptivity evaluation, prior to euploid embryo transfer, improves the success rate in patients with recurrent implantation failure (RIF).

Methods:

Retrospective, observational, cross-sectional study, which included couples undergoing assisted reproduction techniques (2021), who experienced RIF after transfer of good quality, euploid blastocysts (analyzed with PGT-A). Three groups were formed, which depended on the result of ERA: 1) Receptive, 2) Pre-receptive and 3) Early receptive.

Results:

Of the 100% of the patients, 43% presented a normal window of implantation. However, 61.5% showed a higher prevalence of natural killer alterations and 42.8% of thrombophilias. In the case of patients with altered endometrial receptivity, they presented a cumulative implantation rate higher than 70% when correcting controlled ovarian stimulation.

Conclusions:

More than half of the patients with RIF have a displaced implantation window and may benefit from a personalized adjustment of the endometrial stimulation protocol. Thus, couples with idiopathic RIF or associated with thrombophilias and immunological factors, benefit from the study of endometrial receptivity.

KEYWORDS: Recurrent implantation failure, natural killers, ERA, PGT-A and thrombophilias.

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MANUSCRIPT

Introduction

Recurrent Implantation Failure (RIF) is an event that many obstetricians and assisted reproductive specialists may face. Currently, RIF has several definitions; some authors describe it as the impossibility of achieving a clinical pregnancy after the transfer of two good quality embryos in at least three In Vitro Fertilization (IVF) cycles, where the transfers can be performed with fresh or frozen embryos (total, 6 embryos), or in at least two oocyte donations (total, 4 embryos)[1]. It is also defined as the impossibility of achieving a clinical pregnancy after the transfer of at least 4 good quality embryos in three or more embryo transfers in women under 40 years of age[2]. The implantation process depends on 2 main components: 1) healthy embryos with implantation potential, and 2) a receptive endometrium suitable for implantation[3,4,5,6]. In order for implantation to be successful, both the embryo and the endometrium produce mediators (integrins, MUC 1, COX-2, HOXA 10, LIF, calcitonin, etc.) that, together with cytokines produced by lymphocytes [T, B, macrophages and Natural Killer (NK) cells] of the maternal immune system, promote this process[3,4,5,6]. Factors associated with implantation failure include anatomical factors (uterine anatomical abnormalities and thin endometrium), pelvic factors (altered expression of adhesive proteins, hypercoagulable state, immunological alterations), embryonic factors (genetic abnormalities, alterations in hatching (zona pellucida), embryo culture and transfer), energy deficiency and male factors[7,6,8].

The endometrium is a dynamic tissue that undergoes multiple changes during the menstrual cycle, for example it responds to hormones produced in the ovary as well as to paracrine secretions. In this regard, paracrine and endocrine secretions control the gene expression of endometrial cells. The proliferative phase is controlled by estrogens, allowing the proliferation of stromal cells and glands, as well as the elongation of the spiral artery. Post ovulatory progesterone (P4) causes secretory changes and, therefore, the endometrium acquires a receptive phenotype that allows blastocyst implantation. This period of endometrial receptivity (ER) is known as the "window of implantation (WOI)" and occurs between day 19 and 20 of the menstrual cycle[9,10]. Currently, there are no objective and accurate methods to evaluate the ER, which, together with its lack of evaluation in infertile patients undergoing assisted reproductive techniques (ART), could lead to a decrease in the implantation rate because the focus is mainly directed to embryo development and embryo quality[11].

For this reason, in recent years techniques have been developed that more accurately indicate the WOI, thereby improving the success rate of assisted reproduction clinics. Transcriptomics or RNA sequencing emerged as a powerful tool for the clinical diagnosis of cancer, cardiovascular pathologies and neurodegenerative diseases, among others[12]. However, in the case of infertility, it focuses on improving the implantation rate, which is based on the genetic information obtained from the human endometrium and generated during the last 18 years, allowing the development of endometrial receptivity analysis (ERA), which is composed of the evaluation of 248 genes analyzed by next generation sequencing (NGS) and coupled to a computational predictor that allows to appreciate the ER status to identify the WOI [13].

Therefore, the aim of this study is to analyze whether the evaluation of endometrial receptivity prior to embryo transfer improves the success rate in patients with recurrent implantation failure, to whom embryos with good embryo development, good quality and without aneuploidy [Preimplantation Genetic Testing for Aneuploidies) PGT-A] have been transferred.

Material and Method

Retrospective, observational, cross-sectional study that evaluated the results of ART in couples with recurrent implantation failure, obtained in 2021 at the Pronatal clinic located inside the Hospital Bité Médica in Mexico City. Thirty-seven women over 18 years of age, who experienced RIF after transfer of good quality, euploid blastocysts (analyzed with PGT-A), were included. Patients who failed to have a clinical pregnancy after transferring three good quality embryos in different single embryo transfers, either own or donated, were diagnosed with RIF. Three groups were formed which depended on the outcome of ERA: 1) Receptive, gene expression profile is compatible with normal receptive endometrium, it was recommended to perform blastocyst(s) transfer following the same endometrial preparation protocol used during the same ERA analysis, 2) Pre-receptive, gene expression profile could indicate a displacement of WOI, it is recommended to delay the transfer of blastocyst(s) with respect to the time when the endometrial biopsy was taken and 3) Early receptive, this gene expression means that the endometrium is at the beginning of the receptive stage, it is recommended to delay the transfer of blastocyst(s) with respect to the time when the endometrial biopsy was taken.

From the first consultation, the medical and nursing areas collected age, weight, height and BMI. In addition, data such as Recurrent Pregnancy Loss (RPL), Repeated implantation failure (RIF), obesity,

Premature Ovarian Insufficiency (POI), endometriosis, hypothyroidism, Natural killer (NK) were also collected from the clinical history, Inherite Thrombophilias [IT (MTHFR-C677T and PAI-1 4G>5G, TNF-α G238A, TNFα G308A and LT-α A252G)] homozygous and heterozygous, Insulin Resistance (IR), Frozen Embryo Transfer (FET), implantation rate and clinical pregnancy. In all the alterations found, the patients received treatment.

Endometrial preparation

In order to carry out the endometrial preparation, hormone substitution was performed-which has been used in failed transfers prior to ERA- the administration, in general, was as follows: 4 mg of oral estradiol (Primogyn), starting on day 3 of the menstrual cycle, by day 6 it was increased to 6 mg and by day 11 it reached a maximum of 8 mg daily. Transvaginal ultrasound was used in order to evaluate the pattern and thickness of the endometrium approximately 14 days after menstruation and 800 mg progesterone (Geslutin) was administered, when a trilaminar pattern with a thickness between 8 and 14mm was reached. The initial day of progesterone administration was considered "P+0", and the biopsy was performed after 5 full days of progesterone administration “P+5”.

Endometrial receptivity analysis

All sample were sent to the Igenomix laboratory, there were DNAse treated, and cDNA was obtained by retro- transcription and analysed by targeted RNA-Seq assay on IonTorrent Next Generation Sequencing, for 248 ERA genes in an Ion S5 system. Sequencing files were used as the input of the ERA predictor (Diaz-Gimeno et al., 2011) to quantify the expression of the ERA genes and to assess the endometrial receptivity status of each sample. Briefly, the reads were mapped to the hg19 human genome transcriptome using the STAR read aligner (Dobin et al., 2013). To count the number of reads that could be assigned to each gene, we used the HTSeq tool (Anders et al., 2015) with the union option. The ERA gene counts were used by the prediction model to classify each sample in an endometrial receptivity class: proliferative, pre-receptive, receptive or post-receptive[14].

Preimplantation Genetic Testing for Aneuploidies (PGT-A)

A trophectoderm biopsy was performed in the assisted reproduction laboratory, which was processed and sent to the Igenomix laboratory, where PGT-A was performed using massive sequencing technology (NGS). The Ion ReproSeqTM PGS kit was used for library preparation and the Ion ChefTM System (Thermo Fisher Scientific, USA) was used for 24-chromosome aneuploidy analysis. Sequencing of the libraries was performed with the Ion S5 System sequencer (Thermo Fisher Scientific, USA). For data analysis, the Ion Reporter software is used, which performs the alignment of the reads with respect to the latest version of the human reference genome (hg19) (Thermo Fisher Scientific,USA)[15].

All patients were informed about the use and handling of the collected data, allowing their inclusion in this study. In addition, their anonymity is maintained, as no reference is made to the origin of the information, therefore, only numerical and statistical data (according to each case) are disclosed.

Inclusion criteria: euploid blastocyst transfer, patients with idiopathic recurrent implantation failure, women of reproductive age, endometrial thickness ≥7.

Exclusion criteria: known causes of implantation failure.

Statistical analysis

Patients' age, weight, height and BMI are reported with mean ± standard deviation (SD) and the presence of significant difference between groups was evaluated using Student's T ($p \leq 0.05$). For their part, RPL, RIF, obesity, POI, endometriosis, hypothyroidism, NK, IT and IR were expressed as percentages and the difference between groups was evaluated by performing the Chi-squared test ($p \leq 0.05$). In both cases, the SPSS statistical package, version 25, was used.

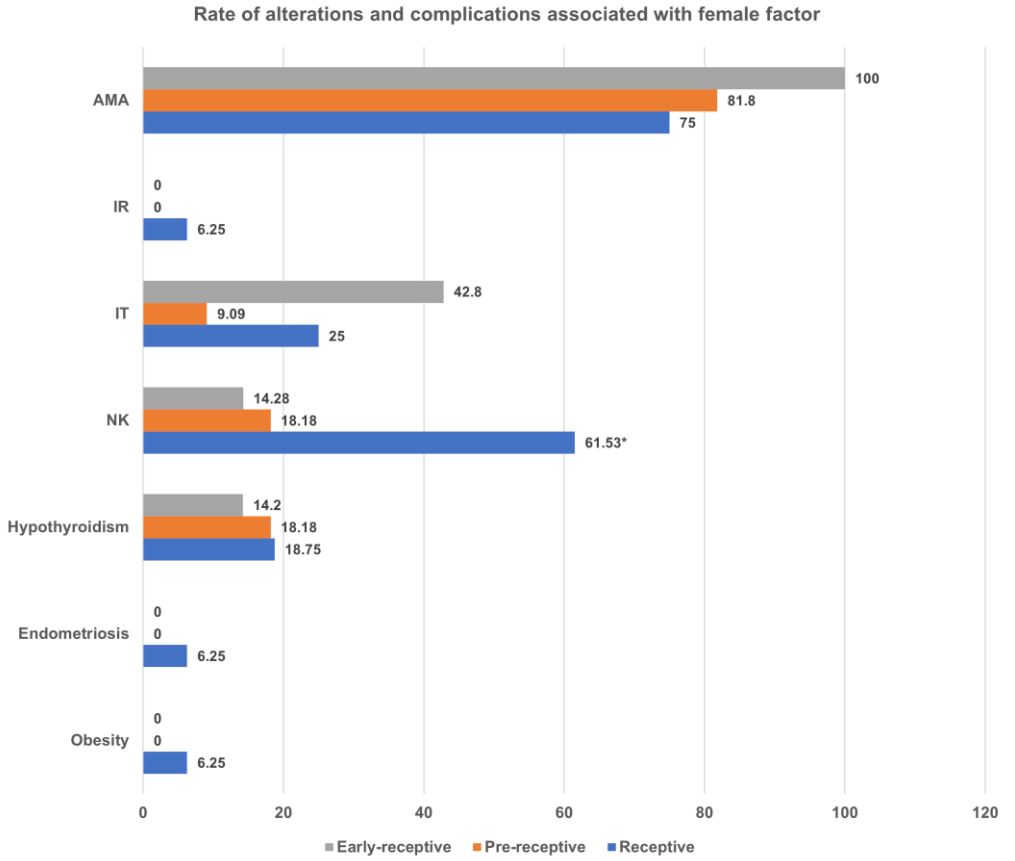
Results

Thirty-seven patients with ERA were included in this study, and the results showed that 16 had a receptive, 11 pre-receptive and 10 early receptive gene profile. When analyzing the anthropometric data, it was observed that only the receptive group presented a statistically significant decrease in maternal age, when compared to the pre-receptive and early receptive groups (36.2±5.6 vs. 38.09±5.5 and 40.4±3.8, $p \leq 0.05$) (Table 1).

	N (%)	Age	Weigth	Size	BMI
Receptive	16 (43.2)	36.2±5.6*	65.9±12.02	1.62±0.06	24.8±4.02
Pre-receptive	11 (29.7)	38.09±5.5	64.1±11.04	1.62±0.06	24.4±3.8
Early receptive	10 (27.02)	40.4±3.8	66.2±6.3	1.60±0.05	25.6±2.6
p	-	≤0.05	>0.05	>0.05	>0.05

Table 1. Maternal anthropometric data.
Student's t-test, statistical difference (p≤0.05).

Regarding the background of the patients, we have that the receptive group presented a significant increase of NK, compared to pre-receptive and early receptive (61.53 vs. 18.18 and 14.2%, p≤005). In parallel, early receptive (42.8%) showed higher prevalence of TI, followed by receptive (25%) and pre-receptive (9.09). In the case of insulin resistance (6.25, 0 and 0%), endometriosis (6.25, 0 and 0%) and obesity (6.25, 0 and 0%), these were only present in the receptive group. In contrast, three groups presented similar prevalence of hypothyroidism (18.75, 18.18 and 14.2%) and POI (12.5, 18.8 and 14.2) (Graph 1).

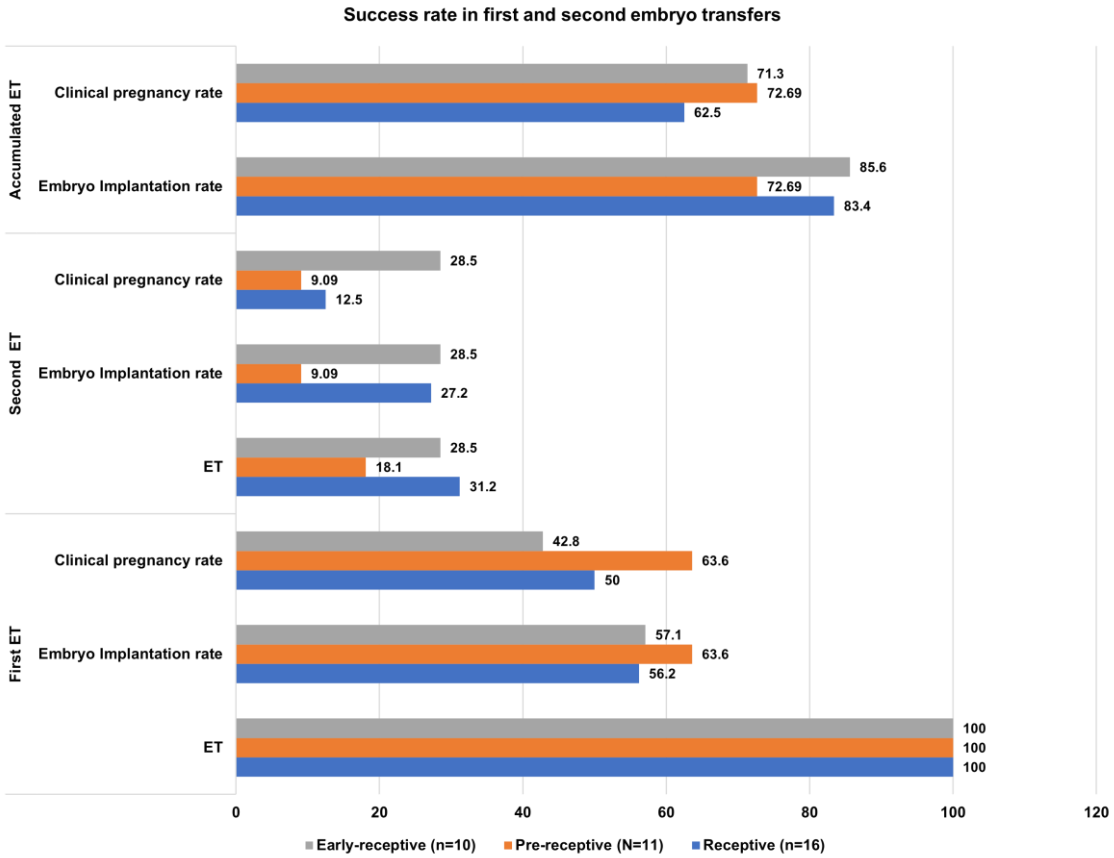


Graph 1. History of alterations and complications. AMA: Advanced Maternal Age, IR: Insulin Resistance, IT: Inherited Thrombophilias and NK: Natural Killers. *Statistically significant difference of Receptive, when compared with Pre-receptive and Early receptive, chi-squared test (p≤0.05).

After performing the first embryo transfer, following the recommendations described in the ERA results sheet, it was found that both "Receptive" (56.3%), "Pre-receptive" (63.6%) and "Early receptive" (57.1) achieved an implantation rate that exceeded 50%. After embryo implantation, the evaluation of the clinical pregnancy rate in this first embryo transfer was maintained in "Pre-receptive" at 63.6%, and decreased non-significantly to 50% in "Receptive" and 42.8% in "Early receptive" (Graph 2).

When a second embryo transfer was carried out in patients who did not achieve embryo implantation in the first transfer, taking as a reference the total number of patients included in the first embryo transfer, the implantation rate was 27.2% in "Receptive", 9.09% in "Pre-receptive" and 28.5% in "Early receptive". These percentages were maintained in "Early receptive" (28.5%) and decreased in "Receptive" (12.5%) and "Pre-receptive" (9.09%) (Graph 2).

Finally, the evaluation of the cumulative implantation rate (results of first + second embryo transfer), was 83.4% in "receptive", 72.69% in "Pre-receptive" and 85% in "Early receptive" (Graph 2). The cumulative clinical pregnancy rate was 62.5% in "Receptive", 72.69% in "Pre-receptive" and 71.3% in "Early-receptive" (Graph 2).



Graph 2. Embryo implantation and clinical pregnancy rate in first and second embryo transfer. ET: Embryo Transfer.

Discussion

In Mexico, 17% of women of reproductive age have infertility problems, which is equivalent to 1.4 million couples in need of assisted reproductive techniques[16,17]. One of the main causes of infertility is implantation failure, which has been associated with embryos with chromosomal alterations. Currently in assisted reproduction clinics, studies have been implemented to identify euploid embryos (without chromosomal alterations) and eliminate most of the genetically abnormal embryos to improve implantation rates and, therefore, the clinical pregnancy rate, such is the case of PGT-A, which in its latest versions uses NGS[18]. Despite this, a proportion of euploid embryos fail to implant, even if no structural pathology is identified in the uterus, which is why in recent years ERA is being implemented[19,20,21].

In this work, "Receptive" the group where patients with the correct WOI and, therefore, receptive endometrial gene expression present high prevalence of patients with NK≥12% in peripheral blood (61.53%), together with the high prevalence of thrombophilias (25%) (Graph 1), this could be the cause of implantation failure, as shown by Luján et al., 2022, in a study that included 54 women with RIF in which 66. 6% presented increased NK in peripheral blood (≥12%) compared to 20% of women in the control group[22]. Similarly, Sacks et al., 2012, the study that included 171 women with RIF, report significant increase of pNK concentrations in mid-luteal phase in RIF group compared to control (11.3 vs 8.7%)[23]. Santillan et al., 2015, in 73 patients with RIF found statistical increase in pNK concentration measured during the mid-luteal phase compared to control (13.4 vs 8.4%)[24]. On the other hand, several authors have associated RIF with the tendency to hypercoagulable states, showing that the mechanisms by which thrombophilias can generate RIF are the alteration of blood flow which decreases endometrial receptivity. In addition, implantation failure has a multifactorial factor and although in a very low proportion in this study the RIF patients presented obesity, endometriosis, hypothyroidism and insulin resistance, all of these have been associated with an increase in implantation failure according to several studies (Figure 1)[25,26,27,28].

As for "Pre-receptive" and "Early-receptive" present statistical decrease in the prevalence of pNK≥12% compared to "Reseptive", ruling it out as the main cause of RIF in these two groups. Similarly, the prevalence of IT in "Pre-receptive" is too low to be associated as the main cause of RIF in this group. Contrary to the aforementioned, TI in "Early-receptive" presented a prevalence of 42.8 %, probably being one of the main causes of RIF. In the case of hypothyroidism, the 3 groups also showed low prevalence (Graph 1).

As can be seen in the previous paragraphs, a higher prevalence of alterations was observed in the history of patients with normal WOI ("Receptive"), which was probably the origin of RIF, and when corrected with personalized treatments, without modifying the endometrial stimulation protocol, a higher implantation rate was achieved: in the first transfer it was 56.2%; and in the second it was 27.2%. Both figures gave a cumulative rate of 83.4% (Graph 2).

However, in "Early receptive" a little more than 42.8% presented an alteration associated with RIF (Graph 1). This group shows how important it is to analyze the RE in patients who have a factor associated with RIF identified, because if they had not undergone ERA, the alteration of the WOI would not have been located nor would the application of endometrial stimulation have been corrected and, consequently, there would be a low prevalence of the implantation rate. On the contrary, the ERA in this group of patients, allowed in this study to identify the exact moment to apply endometrial stimulation, thus achieving an implantation rate in the first embryo transfer of 57.1%, in the second of 28.5%. With a cumulative rate of 85.6% (graph 2). The "Pre-receptive" showed at least 18.8% of some alteration associated with RIF and together with the patients who did not present alterations associated with implantation failure (Graph 1), when correcting the endometrial stimulation protocol as recommended by the ERA report, allowed an implantation rate in the first embryo transfer of 63.6%, in the second of 9.09, with a cumulative rate of 72.6% (Graph 2).

Our results coincide with other studies such as Tan J. et al., 2018, which included 88 patients and found that 44.3% of those who underwent ERA presented displaced WOI (Pre-receptive), achieving after following the recommendations of the ERA report, an implantation rate of up to 73.7%[29]. Similarly, Amin J, et al., 2022, in a group of 219 patients that included embryo transfer from own and donated oocytes, obtained an implantation rate of up to 78% in women with displaced WOI, after correcting endometrial stimulation as recommended in the ERA report[30]. In addition to this, Samadhiya R. et al., 2021 was a study that included 10 women who were non-receptive in ERA, they report an implantation rate of 45.5%, similar to that of the general population undergoing IVF techniques and with normal RE [31]. In addition, in the Pronatal Clinic a prevalence of women with displaced WOI of 56.72% ("Pre-receptive" + "Early receptive") was obtained, higher than that reported in different studies [10, 29].

The importance of this research lies in the fact that it is the first one carried out in a Mexican population. In addition, there is no study that refers to disorders such as obesity, endometriosis, hypothyroidism, pNK, IT and IR, which may be present in patients and which, in many cases, are a factor that influences not to perform ERA, since they are also associated with RIF.

CONCLUSIONS

Our experience shows that more than half of the patients with recurrent implantation failure have displaced implantation window and may benefit from a personalized adjustment of the endometrial stimulation protocol.

Patients with disorders such as obesity, endometriosis, hypothyroidism, natural killers ≥12%, inherited thrombophilias and insulin resistance, associated with recurrent implantation failure, may also have displaced implantation window and will probably not achieve pregnancy if they do not undergo endometrial receptivity study.

Transfer of euploid embryos to a uterus with a normal window of implantation may result in recurrent implantation failure due to altered natural killer levels in peripheral blood and the presence of inherited thrombophilias.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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Surgical sperm extraction versus semen centrifugation: Method of spermatozoa recovery does not correlate with euploidy rates in patients with cryptozoospermia

Alkon-Meadows Tamar

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ABSTRACT

Objective

The aim of this study is to evaluate the rate of embryonic euploidy in blastocysts derived from testicular versus ejaculated sperm in cryptozoospermic patients.

Design

Retrospective cohort analysis.

Material and methods

The study included couples who suffer from Cryptozoospermia and underwent an autologous in vitro fertilization (IVF) with preimplantation genetic testing (PGT-A) cycle(s) from 2014 to 2019. Only cases where oocyte insemination was conducted with intra-cytoplasmic sperm injection (ICSI) were evaluated. Cohorts were separated based on the source of sperm (Ejaculated vs. Testicular (TESE)). Demographic and clinical embryology parameters were compared among cohorts. Student's t-test, Wilcoxon' rank test, chi-square test, and multivariate logistic regression fitted with a GEE model were used for data analysis.

Results

A total of 573 blastocysts derived from 87 IVF/PGT-A cases were included in the study. 74 cases (n= 474 embryos) utilized ejaculated sperm and 13 cases (n= 99 embryos) utilized testicular sperm. No significant differences were found in demographic and stimulation parameters among cohorts. (Table 1) No differences among the ejaculated and testicular cohorts were found in fertilization rate (63.2%; 61.1%, p=0.32); blastulation rate (64.5%; 66.6%, p=0.69); and rate of embryo euploidy (49.7%; 52.1%, p=0.76) respectively. No differences were found in rate of cycle cancellation due to unavailable embryos for TE biopsy (18.9% vs 7.6%, p=0.32).

Conclusions

There is no genomic advantage to surgical sperm retrieval in cryptozoospermic patients.

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MANUSCRIPT

Introduction

Since 1992, intracytoplasmic sperm injection (ICSI) has been used to overcome many causes of male factor infertility, including cryptozoospermia.[1] According to the World Health Organization (WHO) cryptozoospermia is defined as the absence of spermatozoa from fresh preparations but observed in a centrifuged pellet.[2] Due to low sperm count (<103 spermatozoa/mL) observed in the seminal fluid after centrifugation, cryptozoospermic patients require assisted reproductive technology (ART) to achieve a pregnancy.[3] Furthermore, men with cryptozoospermia may suffer from virtual azoospermia.[4] Because of this, many clinicians may consider the use of testicular sperm extraction (TESE) to achieve a better ART outcome.[5,6]

Controversy exists over the use of ejaculate versus testicular sperm for ICSI in cryptozoospermic patients.[5-9] Ejaculate sperm is thought to be more mature than testicular sperm.[10] Nevertheless, there are inherent concerns with the use of ejaculate sperm; the repeated centrifugations needed to identify viable sperm may increase the production of reactive oxidative species affecting the quality of the sperm.[11] Moreover, it has been proposed that sperm could suffer DNA damage due to oxidative stress after the release from Sertoli cells leading to low sperm quality and impaired clinical outcomes.[12-14] If DNA damage detected in ejaculated spermatozoa begins after the sperm is release from Sertoli cells, it can be hypothesized that sperm recovered directly from the testis could be less affected by this pathological process when compared with ejaculated sperm.

On the other hand, TESE has shown to have debatable benefits over ejaculate sperm,[5-9] and it carries risks of surgical complications and long-term adverse effects including hypoandrogenism.[15,16] Additionally, previous studies have described a correlation between testicular extracted sperm and spermatic aneuploidy in patients with non-obstructive azoospermia.[17] However, there are currently no peer reviewed publications associating higher aneuploidy rates with cryptozoospermia.

Given the lack of information regarding the possible causes for suboptimal outcomes patients with cryptozoospermia, and the possible relationship of embryonic aneuploidy in embryos derived form TESE, we sought to determine whether the embryonic euploidy rate differs in blastocysts derived from testicular versus ejaculated sperm in cryptozoospermic patients.

Materials and Methods

Study design and patient population

This retrospective, single center study included all cryptozoospermic patients who underwent in vitro fertilization (IVF) with ICSI and preimplantation genetic screening for aneuploidy (PGT-A) at RMA NY using next generation sequencing, from 2014 through December 2019. Cases of patients harboring chromosomal rearrangements, undergoing preimplantation genetic testing for monogenic defects (PGT-M) and/or using donor gametes were excluded from the analysis. Cohorts were segregated based on the source of sperm (ejaculated versus testicular). Demographic characteristics such as age, BMI (body mass index), ovarian reserve metrics were collected. Cycle characteristics and embryologic data, including number of mature oocytes (MII)fertilization rate, blastulation rate (total number of viable blastocysts over the total number of fertilized oocytes), embryo quality and ploidy rates (number of euploid/aneuploid/indeterminate blastocysts over the number of biopsied blastocysts) were compared between cohorts.

Stimulation protocol

Patients underwent controlled ovarian hyperstimulation (COH) for IVF as previously described.[18] Briefly, the COH protocol was selected at the discretion of the reproductive endocrinologist and involved the administration of follicle-stimulating hormone (FSH) and human menopausal gonadotropin (hMG) with a gonadotropin-releasing hormone (GnRH) agonist downregulation protocol with leuprolide acetate (Lupron, AbbVie Inc., North Chicago, IL), a GnRH antagonist protocol (Ganirelix Acetate, Organon USA Inc., Roseland, NJ or Cetrotide, EMD Serono, Rockland, MA), or a microflare protocol with leuprolide acetate (Lupron, AbbVie Inc., North Chicago, IL). These protocols have been described previously.[18] Follicular development was monitored using transvaginal ultrasonography. When at least two follicles reached 18 mm in diameter, final oocyte maturation was induced with either hCG (5000–10,000 IU, Novarel, Ferring Pharmaceuticals, Parsippany, NJ, USA), recombinant human chorionic gonadotropin (250–500 µg, Ovidrel, EMD Serono, Rockland, MA) or, in high responders at risk of ovarian hyperstimulation syndrome undergoing a GnRH antagonist protocol, a dual trigger with 2 mg of leuprolide acetate and 1000 IU of Hcg or leuprolide acetate alone. Thereafter, patients underwent vaginal oocyte retrieval under transvaginal ultrasound guidance 36h after oocyte maturation was triggered.

Methodology of the TESE Procedure

Patients with cryptozoospermia underwent testicular sperm retrieval as previously described.[20] Briefly, after stabilization of the testicle, a small incision in the testicle's midportion was performed, cutting through the scrotal skin, tunica vaginalis, and albuginea. A substantial piece of the extruding testicular tissue was cut with small scissors, washed with medium to remove blood traces, and placed in a Petri dish. Testicular tissue was vigorously fragmented and minced using two glass slides and immediately examined under the inverted microscope for the presence of spermatozoa in a wet preparation. Once spermatozoa were found, the surgical procedure was terminated. If spermatozoa were not observed, additional biopsies were taken from different areas of the same testicle and also from the contralateral one.

Laboratory procedures

Embryo Culture

All metaphase II (MII) oocytes underwent intracytoplasmic sperm injection (ICSI). Embryos were cultured to the blastocyst stage as previously described (Hernandez-Nieto, et al. 2019). On day 3 of embryo development, all embryos underwent laser-assisted zona hatching by creating a 25–30 µm opening in the zona pellucida with a 200– 300 ms pulse using ZILOS-tk Laser (Hamilton Thorne Biosciences, MA, USA) to facilitate posterior trophectoderm herniation. Blastocyst trophectoderm biopsies were performed on day 5-7 of development, contingent upon morphologic eligibility (Modified Gardner Scoring system).[19] Biopsy was performed as described previously.[18] The biopsy samples were placed in hypotonic wash buffer and submitted for analysis. Embryos were vitrified after the biopsies. Five to seven cells were analyzed by next generation sequencing (NGS) in order to determine chromosome, copy number and assigned to the following categories: euploid, aneuploid or inconclusive by the reference laboratory (during the study period mosaicism was not yet reported).

Outcome measures

The primary outcome was ploidy rate in blastocysts derived from testicular versus ejaculated sperm in cryptozoospermic patients, defined as the number of euploid and/or aneuploid blastocysts over the total number of biopsied blastocysts. Secondary outcome measures included fertilization rate, blastulation rate (total number of viable blastocysts over the total number of fertilized oocytes), and number of biopsied embryos.

Statistical analysis

Descriptive data was compared by Student's t-test, Wilcoxon' rank test and chi-square test when appropriate. The results were expressed as percentages, means and SDs with Clopper–Pearson binomial 95% CI. Adjusted odds ratios (OR) with 95% CI were calculated using multivariate logistic regression analyses to adjust for confounding variables. Logistic regression models were fitted with generalized estimating equations (GEE) to account for patients that underwent multiple cycles. Statistical analyses were performed using SAS version 9.4 (SAS institute Inc., Cary, NC, USA). All p-values were two-sided and were considered significant if less than 0.05.

Regulatory approval

This retrospective study was approved by the Icahn School of Medicine at Mount Sinai Institutional Review Board, Inc.

Results

A total of 573 blastocysts derived from 87 IVF PGT-A cases were included in the study. 74 cases (n= 474 embryos) utilized ejaculated sperm and 13 cases (n= 99 embryos) utilized testicular sperm. No significant differences were found in demographic and cycle characteristics among cohorts. (Table 1).

	Ejaculated sperm cycles		Testicular sperm cycles		
	N=74		N=13		
	Mean	SD	Mean	SD	
Male patient age (years)	39.77	7.48	42.82	7.26	0.17
Female patient age (years)	36.13	4.36	36.98	5.29	0.53
BMI (kg/m2)	24.73	4.53	23.91	5.10	0.55
Baseline FSH (IU/mL)	6.01	4.03	4.80	2.46	0.36
Antimullerian hormone (ng/ml)	2.60	3.45	3.05	2.35	0.69
Baseline Antral Follicle count	10.81	6.05	12.70	3.92	0.34
Surge E2 (pg/mL)	2055.10	1036.86	2490.62	1215.28	0.17
Mature MII oocytes	10.12	6.50	12.46	7.15	0.24
Fertilized oocytes	6.41	4.57	7.62	6.28	0.40
Total blastocysts / Cycle	4.14	4.01	5.08	5.16	0.45
Biopsied embryos /Cycle	3.65	2.95	5.75	4.83	0.26
Euploid embryos/ Cycle	1.81	2.06	3.00	3.07	0.16
Previous Oocyte Retrievals	0.69	1.80	1.00	1.22	0.55
	N	%	N	%	
Cancelled cycles / No embryos for Biopsy	14/74	18.9	1/13	7.6	0.32
Fertilization rate	474/749	63.2	99/162	61.1	0.61
Blastulation rate	306/474	64.5	66/99	66.6	0.69
Blastocyst biopsied/ Non biopsied Rate	201/306	65.6	46/66	69.6	0.53
Euploidy rate	100/201	49.7	24/46	52.1	0.76

Table 1. Demographic cycle characteristics and laboratory outcomes of couples who suffer from cryptozoospermia and underwent an autologous IVF with PGT-A.

Note: Data presented as mean, percentages and standard deviation, unless stated otherwise. Abbreviations: BMI, body mass index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; MII, metaphase II. Significance established at $p < .05$.

No differences were found in fertilization rate (63.2%; 61.1%, p=0.32); blastulation rate (64.5%; 66.6%, p=0.69); and rate of embryo euploidy (49.7%; 52.1%, p=0.76) among cohorts. Finally, no differences were found in rate of cycle cancellation due to unavailable embryos for TE biopsy (18.9% vs 7.6%, p=0.32). (Table I)

After adjusting for female and male patient's age, BMI, AMH, and number of biopsied embryos, there were no association with surgical extracted sperm and lower odds of embryo euploidy (OR 0.69, CI95% 0.11-4.3, p=0.69).

Discussion

The results of this analysis suggest that there is no association with surgical extracted sperm and lower rates of embryonic euploidy. Whereas prior studies have investigated reproductive outcomes of fresh ejaculate or TESE and the transfer of unscreened embryos in patients with cryptozoospermia, this study is among the first to focus on the influence of the sperm source (from testicular versus ejaculated sperm) over the embryonic euploidy rate in cryptozoospermic patients.

When comparing ICSI outcomes using testicular versus ejaculate sperm in patients with cryptozoospermia in fresh embryo transfers, studies

have yield divergent result.[20,21] O'Connell et al. demonstrated ejaculated sperm to be more optimal than testicular sperms in cryptozoospermia patients and suggested that fertilization rates are related to sperm maturation.[20] Conversely, Cui X et al. demonstrated that the use of testicular sperm achieved better embryonic quality and IVF outcomes than ejaculate sperm.[21] Few studies have compared the fertility outcome of ejaculate with testicular sperm cells. Weissman et al reported a series of 4 couples with male factor infertility and multiple failed IVF/ICSI cycles with poor embryo quality and repeated implantation failure using motile ejaculatory sperm cells. The use of fresh testicular sperm cells resulted in better embryonic quality and pregnancies in all cases.[22] Contrary to Weissman findings, our study found similar laboratory outcomes when utilizing both ejaculated or testicular sperm and ICSI.

Normal chromosomal composition is a primary driver of embryonic competence and reproductive success in patients undergoing ART. It is well established that the most important factor to achieve a euploid embryo is the oocyte's age.[23] However, the paternal genome also plays a crucial role.[24] Because of this, there has been growing concerns regarding the possible chromosomal anomalies in offspring of men with severe male infertility.[24] Particularly in embryos derived from testicular versus ejaculate sperm in spite of a young female partner.[25] Even if the infertile male is chromosomally normal in his peripheral lymphocytes, a meiotic disruption may generate high rates of sperm chromosome abnormalities. Because of this, many researchers suggest evaluating the chromosomal complement of the spermatozoa in patients with severe male factor infertility and normal karyotypes.[26] Multiple studies utilized fluorescence in situ hybridization (FISH) to assess the genetic composition of ejaculate sperm in comparison with surgically retrieved spermatozoa. One of the earliest reports studied chromosomes X, Y, 18 in the spermatozoa of 34 men with severe male factor infertility. The authors claimed that testicular spermatozoa presented higher rates of chromosomal aneuploidy in comparison with ejaculate sperm (19.6% vs 13%).[27] In light of this, Cheung et al. compared sperm aneuploidy rates in ejaculated and testicular spermatozoa in the same individuals using FISH and NGS. After evaluating 9 chromosomes, the study reported that the total aneuploidy of surgically retrieved spermatozoa are comparable to that of ejaculated spermatozoa, corroborating that the use of testicular sperm is safe and does not increase aneuploidy rates.[28] Our study findings are similar as we found no association with surgical extracted sperm and lower odds of embryo euploidy (OR 0.69, CI95% 0.11-4.3, p=0.69).

Our study distinguishes itself as it was performed at a single, high-volume academic center with a team of embryologists all uniformly trained, thereby reducing the inherent variability that may arise from multicenter studies. Patients with recognizable risk factors for poor embryonic development, such as parental chromosomal rearrangements, were excluded from the analysis, thus making our findings more generalizable. Aside from a large cohort, we use clinically validated PGT-A techniques to assess the rates of embryonic ploidy for all embryos analyzed, ensuring uniformity within the embryonic genetic results.

Notwithstanding our best efforts to avoid biases, some shortcomings and limitations exist in the analysis. The most notable limitation is its retrospective design, which increases the chance of selection bias. Furthermore, the number of patients that underwent a TESE is limited, However, the retrieval of testicular sperm mandates a surgical intervention and embedded risks. In light of the lack of data about the preferable source of sperm cells for ICSI in patients with cryptozoospermia, it would be unethical to design such a prospective research rather than to first use ejaculated sperm.

CONCLUSION

To our knowledge, this study is the first to evaluate the rate of embryonic euploidy in blastocysts derived from testicular versus ejaculated sperm in cryptozoospermic patients. Our analysis shows that there is no genomic advantage to surgical sperm retrieval in cryptozoospermic patients. Furthermore, we demonstrated that the use of testicular or ejaculate spermatozoa for ICSI can compensate for the reproductive disadvantage associated with the semen parameters of patients with cryptozoospermia. These data can be used to counsel patients about the chromosomal composition of embryos and ART outcomes derived when cryptozoospermia is encountered and to reassure them that the method of sperm collection prior to insemination via ICSI will not influence their IVF clinical success. Further randomized prospective studies should be performed in order to generate personalized and evidence-based recommendations for couples facing cryptozoospermia.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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Embryo Transfer is the last Frontier for Deep Machine Learning & Artificial Intelligence in Medically Assisted Reproduction (MAR)

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ABSTRACT

Embryo Transfer is the last Frontier for Deep Machine Learning & Artificial Intelligence in Medically Assisted Reproduction (MAR). In the last five years, nearly every aspect of an IVF cycle has been investigated by artificial intelligence(AI) & deep machine learning (ML), including sperm morphology, sperm identification, identification of empty or oocyte containing follicles, prediction of embryo cell stages, prediction of blastulation from oocytes, scoring blastocyst quality, prediction of euploid blastocysts and live birth from blastocysts, improving the embryo selection process, and for developing algorithms for optimal IVF stimulation protocols. Moreover, AI-based methods can be implemented for other clinical aspects of IVF, such as assessing patient reproductive potential and individualizing gonadotropin stimulation protocols. As AI has the capability to analyze "big" data, the ultimate goal will be to apply AI tools to the analysis of all embryological, clinical, and genetic data in an effort to provide patient-tailored treatments. Embryo Transfer is the only step of IVF that is outside the realm of AI & ML today. Embryo Transfer success is presently human skill dependent and deep machine learning may one day intrude into this sacred space with the advent of specialized humanoid robots. Embryo transfer is arguably the rate limiting step in the sequential events that complete an IVF cycle. Many variables play a role in the success of embryo transfer, including catheter type, atraumatic technique, and the use of sonography guidance. In this clinical review we will cover the contemporary research goals of AI & ML as well as the variables influencing Embryo Transfer success.

KEYWORDS: Artificial Intelligence (AI), IVF, Embryo Transfer.

MANUSCRIPT

Introduction

An increasing trend in research funding towards artificial intelligence (AI) & deep machine learning (ML) has re-animated huge expectations for future applications. According to the earliest proponents of AI in IVF, embryo evaluation and selection embody the aggregate manifestation of the entire in vitro fertilization (IVF) process. It aims to choose the "best" embryos from the larger cohort of fertilized eggs, the majority of which will be determined to be not viable either because of abnormal development or due to chromosomal abnormalities. Indeed, it is generally acknowledged that even after embryo selection based on morphology, time-lapse microscopic photography, or embryo biopsy with preimplantation genetic testing (PGT-A), implantation rates in the human are difficult to

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predict. Recently, several artificial intelligence (AI)-based methods have emerged as objective, standardized, and efficient tools for evaluating human embryos[1,2]. Artificial Intelligence (AI) and Machine Learning (ML) are clearly emerging technologies in Medically Assisted Reproduction (MAR) and would benefit from early application of reporting standards.

Culturing of human embryos in optimal conditions is crucial for a successful in vitro fertilization (IVF) program. In addition, the capacity to assess and grade embryos correctly will allow for transfer of the potentially 'best' embryo first, thereby shortening the time to pregnancy. It will also encourage and facilitate the implementation of single embryo transfers (SET), thereby increasing maternal & fetal safety. Time-lapse technology (TLT) introduces the concept of stable culture conditions, in connection with the possibility of continuous viewing and documenting of the embryo throughout its development. However, so far, even when embryo quality scoring is based on large datasets, or when using TLT, the morphokinetic scores are still mainly based on subjective and intermittent annotations of morphology and set timings. Also, the application of strong algorithms for widespread use is hampered by large variations in culture conditions between individual IVF laboratory protocols. Zou et al's recent study emphasizes that clinical features can largely improve embryo prediction performance, and their combination with TLM parameters is robust to predict high-grade euploid blastocysts. The models for ploidy prediction, however, were not highly predictive, suggesting they cannot replace preimplantation genetic testing currently[1]. New methodology, involving deep machine learning enriched with multi-centric clinical data, where every image from the time-lapse documentation is analyzed by an algorithm, looking for patterns that link to outcome, may in the future provide a more accurate and non-biased embryo selection process[2].

Embryo transfer is a key stage in IVF, in which the skillset of the gynecologist itself determines the outcome. Few advances have occurred in the last few decades with regard to the actual procedure of Embryo Transfer. Studies conducted thus far have focused on factors and interventions taking place before, during (with simulators) and after this procedure. Numerous methods, including the use of ultrasound guidance for proper catheter placement in the endometrial cavity, have been suggested as more effective techniques of embryo transfer[3-5]. The moot question is which factors and interventions have thus far been proven to increase pregnancy rates and live birth rates. In this article, we will review the evidence relating to the most important variables influencing embryo transfer techniques in a systematic manner with a view to provide practical recommendations to practitioners involved in medically assisted reproduction (MAR).

Discussion

Why is Embryo Transfer (ET) Human skill-dependent?

Many patient and embryo factors influence the outcome of assisted reproductive technology (ART) treatment. The predictors for a successful ART cycle include female age, ovarian reserve, embryo quality, endometrial receptivity, and the embryo transfer (ET) technique. ET, the final step of ART, has recently been noted as a crucial step affecting ART success. Factors affecting pregnancy rates following ET include either abdominal or transvaginal ultrasound guidance, ease of passage of ET transfer, catheter type and build, the transfer technique, the catheter-loading technique, blood or mucus inside or outside the catheter lumen, retained embryos, mock transfer, the physician's training & experience, and catheter tip location. Despite the lack of consensus regarding the optimal ET technique, it is generally recommended that during ET, the disruption of the endometrium and the induction of uterine contractions should be avoided[5]. The exposure of embryos to the ambient conditions should be minimized, and the embryo(s) should be placed at a pre-determined optimal position within the fundal region of the uterine cavity.

Numerous published papers now document that the ET procedure has an impact on pregnancy and delivery rates after IVF. Difficult transfers should be avoided, as they reduce implantation and pregnancy rates. A total of 7,714 ETs were analyzed by Kava-Braverman et al[6]. The clinical pregnancy rate (CPR) was significantly higher in the cases of easy ET compared with difficult ET (38.2% vs. 27.1%). Each instrumentation needed to successfully deposit the embryos in the fundus involved a progressive reduction in the CPR: use of outer catheter sheath (odds ratio [OR] 0.89; 95% confidence interval [CI] 0.79-1.01), use of Wallace stylet (OR 0.71; 95% CI 0.62-0.81), use of tenaculum (OR 0.54; 95% CI 0.36-0.79). Poor ultrasound visualization significantly diminished the CPR. The CPR decreased progressively with the use of additional maneuvers during ET[6].

Importance of training Physicians for Embryo Transfer

Training residents and fellows is the single most important factor in contemporary reproductive medicine that separates man from machines. A recent study by McQueen et al revealed striking differences between fellowship programs regarding the adequacy of ET technique training; nearly one-half of third-year fellows had performed fewer than ten ETs. With appropriate supervision & training, there is no difference in live birth rate between ETs performed by fellows and attending physicians[7]. The authors suggested that efforts should be made to address barriers and set minimums for the number of transfers performed during fellowship[7].

Ramaiah et al assessed the value of the American Society for Reproductive Medicine Embryo Transfer Certificate Course in confidence and skill building for

performing a live embryo transfer (ET)[8]. The main study outcomes included ET simulation scores of all exercises analyzed at various points of the training and self-assessed confidence before and after the completion of the Embryo Transfer Certificate Course based on a 6-point Likert scale and association of both with extent of prior live ET experience and year of the Reproductive Endocrine (REI) fellowship. The American Society for Reproductive Medicine Embryo Transfer Certificate Course data analysis demonstrated the effectiveness of simulator-based ET training for REI fellows across their 3 years of training, regardless of prior experience with live ET[8].

What are the main variables according to contemporary Evidence Based Medicine (EBM) influencing Embryo Transfer success?

Depth of Placement of Embryos under Ultrasound guidance at ET

Placing the embryos at 10-20 mm from the fundus and at an endometrial thickness of more than 7mm is recommended for good clinical pregnancy outcomes[9]. Davar et al's recent study suggested that the depth of intrauterine embryo placement at a distance of 25 ± 5 mm below the fundal endometrial surface give better IVF results[10].

Pacchiarotti et al's results also suggest that the depth of embryo replacement may be an important variable in embryo transfer technique[11]. The authors recommend transferring at least more than 10mm away from fundus. Pregnancy rates and ongoing PRs are higher if the embryos are replaced at a distance >10 mm from the top of the fundus. In addition, because significantly more embryos were replaced in cycles where the transfers occurred at a distance of >20 mm, a distance>10 mm to <20 mm seems to be the best site for embryo transfer to achieve higher PRs[11].

The objective of Santos et al's study was to determine the influence of the embryo placement depth on the endometrial cavity in relation to the pregnancy rates, after frozen-thawed embryo transfers performed under ultrasound guidance[12]. The patients were classified according to three variables: <10mm, 10 to 15mm and >15mm. Clinical and ongoing pregnancy rates were higher in the 10-15mm and >15mm Groups, when compared to the <10mm Group; there was no statistical difference between the groups in terms of miscarriage and live birth rates. They performed a subsequent analysis, using the same sample of patients, comparing only the <10mm and ≥10mm variables. The ≥10mm Group had better reproductive outcomes, with higher clinical and ongoing pregnancy rates. The authors concluded that pregnancy rates are influenced by the embryo transfer site, and better results can be achieved when the tip of the catheter is placed in the central area of the endometrial cavity, especially when the distance from the endometrial fundus is >10mm[12].

In Ivanovski et al's study, the transfer catheter was advanced to a defined distance from the uterine fundus, up to the point estimated for transfer: 10 +/- 2.5 mm and 15 +/- 2.5 mm respectively in A and B group. Analysis of their results demonstrated that pregnancy rate was significantly influenced by transfer distance from the fundus where the pregnancy rate decreases from 46.2% in group B to 28.8% in group A ($p < 0.05$)[13].

Speed of injection at the time of ET

Catheter injection speed affects depth and placement of the embryo into the uterine cavity and is shown to be highly variable in, and between, subjects in a manually performed embryo transfer. In an effort to standardize the injection speed during embryo transfer, Caanen et al developed an automated transfer pump: the pump-regulated embryo transfer (PRET) device[14]. In a randomized controlled trial, they aimed to investigate if standardization of the injection speed and pressure with this PRET results in a better controlled positioning of the transferred embryo(s). Five hundred ninety-nine embryo transfer cycles were randomly assigned to the PRET or manual transfer. Positioning of the embryo(s) into the uterine cavity was measured with ultrasound. The PRET device generated a significantly smaller variance of the positioning of the embryo(s) into the uterine cavity. This resulted in an ongoing pregnancy rate of 21% in the PRET versus 17% in the manual ($p = 0.22$) transfer group. The PRET results in better controlled positioning of the embryo(s), and it also gives the opportunity to standardize embryo transfer[14].

Mo et al set up a study to evaluate the location of transferred embryos under various parameters during embryo transfer in in vitro fertilization (IVF) by applying an in vitro experimental model for embryo transfer (ET)[15]. Mock ET simulations were conducted with a lab model of the uterine cavity. Embryo transfer catheter was loaded with a sequence of air and liquid volumes as well as development-arrested embryos donated by patients. The transfer procedure was recorded using a high-definition video camera. The medium speed-injected embryos were usually located in the static region while fast- and slow-speed injected embryos were mostly localized at the uterine fundus and the cervical region, respectively. The probability of embryo separation from the air-bubble interface increased from 11.1% in slow injection cases to 29.6% and 48.1% in the medium and fast injection cases, respectively. The authors suggested that faster injection of embryos into a retroverted uterus usually results in the embryo dissociating from the air bubble[15].

Measurement of Utero-cervical length before ET

Bakas et al examined the accuracy of embryo transfer based on the previous measurement of the utero-cervical length[16]. All patients had transvaginal ultrasound measurement of utero-cervical length prior to embryo transfer and measurement of embryo distance (intrauterine air bubbles) from fundal surface

of uterine cavity and internal cervical os immediately after embryo transfer. Primary outcome was to estimate the accuracy of embryo transfer based on the measurement of the embryo distance from middle of uterine cavity after embryo transfer and secondary outcome was to assess the effect of embryo distance from uterine fundus and internal cervical os to clinical pregnancy rate. The study concluded that ET by a single operator with the previous measurement of utero-cervical length and estimation of embryo transfer position will be very accurate[16].

Preload or Afterload at ETs

Preload direct ETs with soft catheters under ultrasound guidance is currently considered the best procedure[17-18]. A prospective randomized unblinded controlled clinical trial by Levi Setti et al, included 352 ultrasound-guided ETs assigned to either direct ET or afterload ET[19]. The primary outcome was the rate of difficult or suboptimal transfers defined as: advancement of the outer sheath (specific for the direct transfer), multiple attempts, use of force, required manipulation, use of a stylet or tenaculum, dilatation, or use of a different catheter. The secondary outcome was clinical pregnancy rate. The rate of difficult transfers was significantly higher in the direct ET group compared with the afterload ET group, although a wide variation was observed among operators[19].

Retention of Embryos at ET

The retention of the embryo in the transfer catheter after embryo transfer (ET) during in vitro fertilization is a common feature, encountered by even the most experienced IVF physicians, and embryos retained in the embryo transfer catheter or within its sleeve require a repeat embryo transfer[20-21]. The exact mechanism of embryo retention has not been explained. Therefore, Kozikowska et al's study aimed to investigate the mechanism of embryo retention in the catheter during embryo transfer by using a transparent uterus model equipped with pressure sensors and a video recorder[22]. Their results indicated that pressure changes in the uterine cavity during ET can influence the distribution of the transferred fluid containing the embryo. Under certain conditions, the transferred fluid can flow backward in the catheter, which may lead to retention of the embryo in the catheter.

ET catheter type

An Argentinian study[23] aimed to compare the use of semi-rigid and flexible catheters in terms of pregnancy rate and level of difficulty of the embryo transfer (ET) procedure. The results suggested that a softer catheter may help with difficult ETs[23]. Softer catheters, as also reported by other authors[5,24-25], resulted in better implantation rates.

ET catheter rotation during withdrawal

Literature suggested that catheter rotation during an ET could discharge mucus entrapped in the embryo to neutralize embryo displacement. The aim of Eftekhar et al's study was to compare the outcome of frozen embryo transfer (FET) based on catheter rotation during withdrawal[26]. Patients were divided into two groups (n=120/each), including A) the rotation treatment group (360°) that underwent ET using catheter rotation and B) the control group including the subjects who experienced ET with no catheter rotation. Their results demonstrated that catheter rotation during withdrawal increased the implantation rate and clinical pregnancy[26].

Maintenance of tight temperature control during ET

Twenty-nine simulated embryo transfer procedures were carried out across five clinics. A thermocouple probe was used for standardized measurements inside each of the ET catheters to record the changes in temperature that occur in the time period between loading the catheter and placing the catheter in the uterus. In all cases, the temperature at the loaded catheter tip fell rapidly to ambient temperature during transit from the embryo transfer workstation in the IVF lab to the ET procedure room. Considering the sensitivity of the pre-implantation embryo to its immediate environment, the rapid and profound drop in temperatures observed at the catheter tip that houses the embryo during its transit from the IVF laboratory to the uterine environment may affect embryo viability and health[27]. The authors suggested that the issue be addressed to ensure that the tight temperature control continues throughout the embryo transfer procedure and could improve clinical outcomes[27]. We may use pre-heated, thermo-couple embedded ET catheters in the future.

Artificial Intelligence & Machine Learning in IVF

Artificial intelligence (AI) systems have been proposed for reproductive medicine since 1997. Although AI is the main driver of emerging research in reproduction, such as Robotics, Big Data, and internet of things, it will continue to be the engine for technological breakthroughs for the near future[28].

Over the past years, the assisted reproductive technologies (ARTs) have been accompanied by constant innovations. For instance, intracytoplasmic sperm injection (ICSI), time-lapse monitoring of the embryonic morphokinetics, and PGT-A are innovative techniques that increased pregnancy rates. Trending strongly is the use of artificial intelligence (AI) techniques in the embryo or spermatozoa selection.

In vitro fertilization has been regarded as a forefront solution in treating infertility for over four decades, yet its effectiveness has remained relatively low. This could be attributed to the lack of advancements for the method of observing and selecting the most viable embryos for implantation. The conventional morphological assessment of embryos exhibits inevitable drawbacks which include time- and effort-consuming, and imminent risks of bias associated with subjective assessments performed by

individual embryologists. A combination of these disadvantages, undeterred by the introduction of the time-lapse incubator technology, has been considered as a prominent contributor to the less preferable success rate of IVF cycles. Nonetheless, a recent surge of AI-based solutions for tasks automation in IVF has been observed. An AI-powered assistant could improve the efficiency of performing certain tasks in addition to offering accurate algorithms that can inculcate objectivity and decrease subjectivity of the decision-making processes[29].

Predictive modeling has become a distinct subdiscipline of reproductive medicine, and researchers and clinicians are just learning the skills and expertise to evaluate artificial intelligence (AI) algorithms. Diagnostic tests and model predictions are subject to evaluation. The performance of AI models and their potential clinical utility hinge on the quality and size of the databases used, the types and distribution of data, and the particular AI method applied. Additionally, when images are involved, the method of capturing, preprocessing, and treatment and accurate labeling of images becomes an important component of AI modeling. Inconsistent image treatment or inaccurate labeling of images can lead to an inconsistent database, resulting in poor AI accuracy[30].

Artificial Intelligence for Sperm Selection in ART

Although in vitro fertilization (IVF) facilitates the job of spermatozoa, a universally acceptable means of sperm selection is yet to be developed. No objective or reliable sperm quality indicators have been established and sperm selection is, to a great extent, based on subjective qualitative evaluation. An ideal method for sperm selection in ART should be noninvasive and cost-effective and allow the identification of high-quality spermatozoa and yield better outcomes in terms of pregnancy and live birth rates. Microfluidic devices, omics profiling, micronuclei studies, sperm plasma membrane markers, and other techniques, such as Magnetic Activated Cell Sorting (MACS), Raman micro-spectroscopy, and artificial intelligence systems offer fresh approaches to an old problem[31].

Kresch et al identified multiple new promising technologies, each with its own distinct set of benefits and limitations, to enhance chances of sperm retrieval; these include the use of multiphoton microscopy, Raman spectroscopy, and full-field optical coherence tomography during a microdissection-testicular sperm extraction procedure[32]. ORBEYE and ultrasonography technologies can also serve to better visualize areas of sperm production. Finally, artificial intelligence technology can play a role in the identification of sperm and, perhaps, better-quality sperm for use with assisted reproduction.

Artificial Intelligence aided Algorithm for Personalized Ovarian Stimulation for IVF

Letterie & Mac Donald designed a computer algorithm for in vitro fertilization (IVF) management and set up a study to assess the algorithm's accuracy in the day-to-day decision making during ovarian stimulation for IVF when compared to evidence-based decisions by the clinical team[33]. Data were derived from monitoring during ovarian stimulation from IVF cycles. The database consisted of 2,603 cycles (1,853 autologous and 750 donor cycles) incorporating 7,376 visits for training. Input variables included estradiol concentrations in picograms per milliliter; ultrasound measurements of follicle diameters in two dimensions in millimeters; cycle day during stimulation and dose of recombinant follicle-stimulating hormone during ovarian stimulation for IVF. The main outcome measures included accuracy of the algorithm to predict four critical clinical decisions during ovarian stimulation for IVF: [1] stop stimulation or continue stimulation. If the decision was to stop, then the next automated decision was to [2] trigger or cancel. If the decision was to return, then the next key decisions were [3] number of days to follow-up and [4] whether any dosage adjustment was needed. The study described a first iteration of a predictive analytic algorithm that is highly accurate and in agreement with evidence-based decisions by expert teams during ovarian stimulation during IVF[33]. These tools offer a potential platform to optimize clinical decision-making during IVF.

Siristatidis et al[34] proposed a functional in vitro fertilization (IVF) prediction model to assist clinicians in tailoring personalized treatment of subfertile couples and improve assisted reproduction outcome. They penned down the construction and evaluation of an enhanced web-based system with a novel Artificial Neural Network (ANN) architecture and conformed input and output parameters according to the clinical and bibliographical standards, driven by a complete data set and "trained" by a network expert in an IVF setting. The system is capable to act as a routine information technology platform for the IVF unit and is capable of recalling and evaluating a vast amount of information in a rapid and automated manner to provide an objective indication on the outcome of an artificial reproductive cycle.

Can workflow during IVF be facilitated by artificial intelligence to limit monitoring during ovarian stimulation to a single day and enable level-loading of retrievals? A first-iteration algorithm described by Letterie et al was designed to improve workflow, minimize visits and level-load embryology work[35]. This algorithm enables decisions at three interrelated nodal points for IVF workflow management to include monitoring on the single best day, assign trigger days to enable a range of 3 days for level-loading and estimate oocyte number.

Deep Machine Learning aided Implantation Prediction Algorithms using Endometrial Thickness

Endometrial thickness in assisted reproductive techniques is one of the essential factors in the success of pregnancy. Despite extensive studies on endometrial thickness prediction, research is still

needed. Mehrjerd et al aimed to analyze the impact of endometrial thickness on the ongoing pregnancy rate in couples with unexplained infertility using deep machine learning & artificial intelligence based algorithms[36]. A total of 729 couples with unexplained infertility were included in this study. They obtained a 7.7mm cut-off point for IUI and 9.99 mm for IVF/ICSI treatment. The results showed machine learning is a valuable tool in predicting ongoing pregnancy and is trustable via multicenter data for the two subject treatments.

Artificial Intelligence aided Endometrial Transcriptomics Implantation Prediction Algorithms

Combining RNA sequencing data (transcriptomics) with artificial intelligence (AI) led to a clinical revolution in personalizing disease diagnosis and fostered the concept of precision medicine.

Translation of endometrial transcriptomics to the clinic yielded an objective definition of the limited time period during which the maternal endometrium is receptive to an embryo, known as the window of implantation (WOI). In approximately 30% of IVF cycles in which embryo transfer is performed blindly, the WOI is displaced and embryo-endometrial synchrony is not achieved. Extending this application of endometrial transcriptomics, the endometrial receptivity analysis (ERA) test couples next-generation sequencing (NGS) to a computational predictor to identify transcriptomic signatures for each endometrial stage: proliferative (PRO), pre-receptive (PRE), receptive (R) and post-receptive (POST). In this way, personalized embryo transfer (pET) may be possible by synchronizing embryo transfer with each patient's WOI[37].

Artificial Intelligence aided Ultrasound

Artificial Intelligence (AI) has gradually become an effective supplementary method for the assessment of female reproductive function. It has been used in clinical follicular monitoring, optimum timing for transplantation, and prediction of pregnancy outcome. Some literatures summarize the use of AI in this field, but few of them focus on the assessment of female reproductive function by AI-aided ultrasound. Chen et al published the applicability, feasibility, and value of clinical application of AI in ultrasound to monitor follicles, assess endometrial receptivity, and predict the pregnancy outcome of in vitro fertilization and embryo transfer (IVF-ET)[38].

AI based Algorithm using Cytoplasm Movement Velocity of Embryos to predict Blastulation

Can artificial intelligence and advanced image analysis extract and harness novel information derived from cytoplasmic movements of the early human embryo to predict development to blastocyst? In a proof-of-principle study, 230 human preimplantation embryos were retrospectively assessed using an artificial neural network[39]. After intracytoplasmic sperm injection, embryos underwent time-lapse monitoring for 44 h. For comparison, standard embryo assessment of each embryo by a single embryologist was carried out to predict development to blastocyst stage based on a single picture frame taken at 42 h of development. In the experimental approach, in embryos that developed to blastocyst or destined to arrest, cytoplasm movement velocity was recorded by time-lapse monitoring during the first 44 h of culture and analyzed with a Particle Image Velocimetry algorithm to extract quantitative information. Integration of results from artificial intelligence models with the blind operator classification, resulted in 82.6% accuracy, 79.4% sensitivity, 85.7% specificity, 84.4% precision and 81.8% F1 score. This study suggests the possibility of predicting human blastocyst development at early cleavage stages by detection of cytoplasm movement velocity and artificial intelligence analysis[39]. This indicates the importance of the dynamics of the cytoplasm as a novel and valuable source of data to assess embryo viability.

Artificial Vision Morphometry based Implantation Prediction Algorithms

Assessing the viability of a blastocyst is still empirical and non-reproducible nowadays. Chavez Badiola et al developed an algorithm based on artificial vision and machine learning (and other classifiers) that predicts pregnancy from both the morphology of an embryo and the age of the patients[40]. They created a system consisting of different classifiers that is fed with novel morphometric features extracted from the digital microphotographs, along with other non-morphometric data to predict pregnancy. It was evaluated using five different classifiers: probabilistic bayesian, Support Vector Machines (SVM), deep neural network, decision tree, and Random Forest (RF), using a k-fold cross validation to assess the model's generalization capabilities. Their results suggest that the system is able to predict a positive pregnancy test from a single digital image, offering a novel approach with the advantages of using a small database, being highly adaptable to different laboratory settings, and with easy integration into clinical Practice[40].

Loeweke et al performed a series of analyses characterizing an artificial intelligence (AI) model for ranking blastocyst-stage embryos[41]. The primary objective was to evaluate the benefit of the model for predicting clinical pregnancy, whereas the secondary objective was to identify limitations that may impact clinical use. Static images of 5,923 transferred blastocysts and 2,614 Non-transferred aneuploid blastocysts were used in the study. A bootstrapped study predicted improved pregnancy rates between +5% and +12% per site using AI compared with manual grading using an inverted microscope[41]. One site that used a low-magnification stereo zoom microscope did not show predicted improvement with the AI. Visualization techniques and attribution algorithms revealed that the features learned by the AI model largely overlap with the features of manual grading systems. Two sources of bias relating to the type of

microscope and presence of embryo holding micropipettes were identified and mitigated[41].

VerMilyea et al have combined computer vision image processing methods and deep learning techniques to create the non-invasive Life Whisperer AI model for robust prediction of embryo viability, as measured by clinical pregnancy outcome, using single static images of Day 5 blastocysts obtained from standard optical light microscope systems[42]. These studies involved analysis of retrospectively collected data including standard optical light microscope images and clinical outcomes of 8886 embryos from 11 different IVF clinics, across three different countries, between 2011 and 2018.The AI-based model was trained using static two-dimensional optical light microscope images with known clinical pregnancy outcome as measured by fetal heartbeat to provide a confidence score for prediction of pregnancy[42]. The Life Whisperer AI model showed a sensitivity of 70.1% for viable embryos while maintaining a specificity of 60.5% for non-viable embryos across three independent blind test sets from different clinics. These studies demonstrated an improved predictive ability for evaluation of embryo viability when compared with embryologists' traditional morphokinetic grading methods.

Artificial Vision Morphometry based Euploidy Prediction Algorithm

The genetics AI model was trained using static 2-dimensional optical light microscope images of Day 5 blastocysts with linked genetic metadata obtained from PGT-A[43]. The endpoint was ploidy status (euploid or aneuploid) based on PGT-A results. Predictive accuracy was determined by evaluating sensitivity (correct prediction of euploid), specificity (correct prediction of aneuploid) and overall accuracy. When the blind test dataset was cleansed of poor quality and mislabeled images, overall accuracy increased to 77.4%[43]. There was a significant positive correlation between AI score and the proportion of euploid embryos, with very high scoring embryos (9.0-10.0) twice as likely to be euploid than the lowest-scoring embryos (0.0-2.4). When using the genetics AI model to rank embryos in a cohort, the probability of the top-ranked embryo being euploid was 82.4%, which was 26.4% more effective than using random ranking, and ~13-19% more effective than using the Gardner score. The probability increased to 97.0% when considering the likelihood of one of the top two ranked embryos being euploid, and the probability of both top two ranked embryos being euploid was 66.4%. Additional analyses showed that the AI model generalized well to different patient demographics and could also be used for the evaluation of Day 6 embryos and for images taken using multiple time-lapse systems. Results suggested that the AI model could potentially be used to differentiate mosaic embryos based on the level of mosaicism. Results can be used to aid in prioritizing and enriching for embryos that are likely to be euploid for multiple clinical purposes, including selection for transfer in the absence of alternative genetic testing methods, selection for cryopreservation for future use or selection for further confirmatory PGT-A testing, as required. Results demonstrated predictive accuracy for embryo euploidy and showed a significant correlation between AI score and euploidy rate, based on assessment of images of blastocysts at Day 5 after IVF[43].

Time Lapse Technology Based Euploidy Prediction Algorithm

Euploid embryos displaying the normal human chromosomal complement of 46 chromosomes are preferentially selected for transfer over aneuploid embryos (abnormal complement), as they are associated with improved clinical outcomes. Currently, evaluation of embryo genetic status is most commonly performed by preimplantation genetic testing for aneuploidy (PGT-A), which involves embryo biopsy and genetic testing. The potential for embryo damage during biopsy, and the non-uniform nature of aneuploid cells in mosaic embryos, has prompted investigation of additional, non-invasive, whole embryo methods for evaluation of embryo genetic status.

TLT has the characteristics of large amount of data and non-invasiveness. If we want to accurately predict embryo ploidy status from TLT, artificial intelligence (AI) technology is a good choice. A total of 469 preimplantation genetic testing (PGT) cycles and 1803 blastocysts from April 2018 to November 2019 were included in Huang's study[44]. All embryo images are captured during 5 or 6 days after fertilization before biopsy by time-lapse microscope system. All euploid embryos or aneuploid embryos were used as data sets. The euploid prediction algorithm (EPA) was able to predict euploid on the testing dataset with an area under curve (AUC) of 0.80. Their AI model named EPA can predict embryo ploidy well based on TLT data[44].

Time Lapse Technology Based Live Birth Prediction Algorithms

An AI system was created by using the Attention Branch Network associated with deep learning to predict the probability of live birth from 141,444 images recorded by time-lapse imaging of 470 transferred embryos, of which 91 resulted in live birth and 379 resulted in non-live birth that included implantation failure, biochemical pregnancy and clinical miscarriage[45]. The AI system for the first time successfully visualized embryo features in focused areas that had potential to distinguish between live and non-live births. Live birth rate of embryos with good morphological quality and confidence scores higher than 0.341 was 41.1%. The authors concluded that an AI system with a confidence score that is useful for non-invasive selection of embryos that could result in live birth[45].

Based on images of embryos with known implantation data (KID), AI models have been trained to automatically score embryos related to their chance

of achieving a successful implantation. Berntsen et al investigated how a deep learning-based embryo selection model using only time-lapse image sequences performs across different patient ages and clinical conditions, and how it correlates with traditional morphokinetic parameters[46]. The model was trained and evaluated based on a large dataset from 18 IVF centers consisting of 115,832 embryos, of which 14,644 embryos were transferred KID embryos. The fully automated iDAScore v1.0 model was shown to perform at least as good as a state-of-the-art manual embryo selection model. Moreover, full automatization of embryo scoring implies fewer manual evaluations and eliminates biases due to inter- and intraobserver variation[46].

AI Algorithm using Artificial Vision Morphometry & Spent Culture Media & for Live Birth Prediction of Euploid Embryos

Bori et al set up a study aimed to develop an artificial intelligence model based on artificial neural networks (ANNs) to predict the likelihood of achieving a live birth using the proteomic profile of spent culture media and blastocyst morphology[47]. This retrospective cohort study included 212 patients who underwent single blastocyst transfer at IVI Valencia. A single image of each of 186 embryos was studied, and the protein profile was analyzed in 81 samples of spent embryo culture medium from patients included in the preimplantation genetic testing program. The information extracted from the analyses was used as input data for the ANN. Three ANN architectures classified most of the embryos correctly as leading (LB+) or not leading (LB-) to a live birth: 100.0% for ANN1 (morphological variables and two proteins), 85.7% for ANN2 (morphological variables and seven proteins), and 83.3% for ANN3 (morphological variables and 25 proteins). The artificial intelligence model using information extracted from blastocyst image analysis and concentrations of interleukin-6 and matrix metalloproteinase-1 was able to predict live birth with an AUC of 1.0[47]. The model proposed in this preliminary report may provide a promising tool to select the embryo most likely to lead to a live birth in a euploid cohort. The accuracy of prediction demonstrated by this software may improve the efficacy of an assisted reproduction treatment by reducing the number of transfers per patient[47].

Raw Time-Lapse Videos based Deep Machine Learning Implantation Prediction Algorithm

The contribution of time-lapse imaging in effective embryo selection is promising. Existing algorithms for the analysis of time-lapse imaging are based on morphology and morphokinetic parameters that require subjective human annotation and thus have intrinsic inter-reader and intra-reader variability. Deep learning offers promise for the automation and standardization of embryo selection. Tran et al[48] created a deep learning model named IVY, which was an objective and fully automated system that predicts the probability of FH pregnancy directly from raw time-lapse videos without the need for any manual morphokinetic annotation or blastocyst morphology assessment. This study was a retrospective analysis of time-lapse videos and clinical outcomes of 10 638 embryos from eight different IVF clinics, across four different countries, between January 2014 and December 2018.This study is a retrospective analysis demonstrating that the deep learning model has a high level of predictability of the likelihood that an embryo will implant[48].

AI ranked metabolic activity based Implantation Prediction Algorithm

Morphological and morphokinetic analyses utilized in embryo selection provide insight into developmental potential, but alone are unable to provide a direct measure of embryo physiology and inherent health. Glucose uptake is a physiological biomarker of viability and amino acid utilization is different between embryos of varying qualities. Blastocysts with higher developmental potential and a higher probability of resulting in a viable pregnancy consume higher levels of glucose and exhibit distinct amino acid profiles. Embryos were individually cultured in a time-lapse incubator system, and those reaching the blastocyst stage had their morphokinetics annotated and were each assigned a Gardner grade, KIDScore and EmbryoScore. Glucose and amino acid metabolism were measured. Clinical pregnancies were confirmed by the presence of a fetal heartbeat at 6 weeks of gestation[49]. Glucose consumption was at least 40% higher in blastocysts deemed of high developmental potential using either the Gardner grade ($P < 0.01$, $n = 209$), KIDScore ($P < 0.05$, $n = 207$) or EmbryoScore ($P < 0.05$, $n = 184$), compared to less viable blastocysts and in blastocysts that resulted in a clinical pregnancy compared to those that failed to implant ($P < 0.05$, $n = 37$)[49]. Additionally, duration of cavitation was inversely related to glucose consumption ($P < 0.05$, $n = 200$). Total amino acid consumption was significantly higher in blastocysts with an EmbryoScore higher than the cohort median score ($P < 0.01$, $n = 185$). Furthermore, the production of amino acids was significantly lower in blastocysts with a high Gardner grade ($P < 0.05$, $n = 209$), KIDScore ($P < 0.05$, $n = 207$) and EmbryoScore ($P < 0.01$, $n = 184$). These results confirm that metabolites, such as glucose and amino acids, are valid biomarkers of embryo viability and could therefore be used in conjunction with other systems to aid in the selection of a healthy embryo[49].

CONCLUSION

The goal of an IVF cycle is a healthy live-born baby. Despite the many advances in the field of assisted reproductive technologies, accurately predicting the outcome of an IVF cycle has yet to be achieved. One reason for this is the method of selecting an embryo for transfer. Morphological assessment of embryos is the traditional method of evaluating embryo quality and selecting which embryo to transfer.

However, this subjective method of assessing embryos leads to inter- and intra-observer variability, resulting in less than optimal IVF success rates. To overcome this, it is common practice to transfer more than one embryo, potentially resulting in high-risk multiple pregnancies. Although time-lapse incubators and preimplantation genetic testing for aneuploidy have been introduced to help increase the chances of live birth, the outcomes remain less than ideal. Utilization of artificial intelligence (AI) has become increasingly popular in the medical field and is increasingly being leveraged in the embryology laboratory to help improve IVF outcomes[50-65]. And assume we have the perfect AI + ML algorithm for prediction of the correct embryo that will implant and give rise to a live birth, you will still need a skilled gynecologist who will safely and successfully transfer this embryo into the AI+ML ranked receptive uterus.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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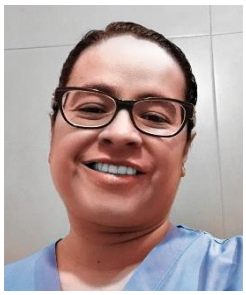
Importance of pre-test genetic counselling in couples undergoing assisted reproductive techniques (ART) and preimplantation genetic testing (PGT).



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ABSTRACT

Seven embryo biopsies from a 33 female and 39 male year-old couple were sent for PGD, all resulted in aneuploid embryos. When analysing the case, we notice that the male had a previous 15-year-old daughter and several semen analyses with fluctuating teratozoospermia from 29% to 100%. Although ICSI and PGD are powerful tools in male factor infertility, it is not recommended in all cases, especially the ones that may be due to reversible causes. Male infertility should be evaluated thoroughly and accompanied by proper genetic studies and genetic counselling so that the couple is offered options suitable for their medical conditions and economy.

KEYWORDS: Genetic counselling, preimplantation genetic diagnosis, assisted reproductive techniques.

MANUSCRIPT

Seven embryo biopsies were sent to us for Preimplantation Genetic Diagnosis (PGD), the clinical notes included ages: female 33 and male 39 years, a masculine factor not specified was pointed out. Most embryos were biopsied on day 3 whereas the last one was biopsied on day 6. Embryo morphology classification was ambiguous and all were aneuploid, as seen in Table 1. About a month later the medical staff at the clinic asked us to reach out to the couple as they were having several doubts about their results. However, the clinical panorama was entirely different from the one referred to us on the paperwork. The first six embryos were from an ovum donor and the last one was from the female, this was not disclosed in the written forms and it is of course, relevant for counselling purposes. The other point of interest is that the male has a previous healthy daughter 15 years old, and has several semen analyses, with fluctuating teratozoospermia from 29% to 100%, Table 2.

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EMBRYO	DAY	MORPHOLOGY CLASSIFICATION	ISCN FORMULA	DIAGNOSIS
(7)	3	8	Complex Aneuploidy	Aneuploid embryo
(9)	3	8	sseq(18)x3^{+}	Aneuploid female
(10)	3	7	sseq(13)x3,(21)x1	Aneuploid male
(11)	3	8	sseq(2,12,21p)x3,(20)x1 mos	Aneuploid female
(12)	3	6	Complex Aneuploidy	Aneuploid embryo
(13)	3	6	Complex Aneuploidy	Aneuploid embryo
(19)	6	BH	sseq(Xp,Xq,21)x3^{+}	Aneuploid female

Table 1

ITEM/DATE	03/2016	03/03/2020	03/14/2020	04/2020	05/2022	Normal value
Volume	6ml	5.1ml	0.5ml	5.8ml	4.5	>1.5ml
pH	6	7.5	9	9	9	7.5
Concentration	93mill/ml	12mill/ml	225mill/ml	53mill/ml	120mill/mil	>15mill/ml
A	NA	0	0	0	0	NA
B	NA	23	45	42	63	NA
C	NA	44	30	14	24	NA
D	NA	33	45	44	14	NA
A+B	NA	23	NA	42	86	NA
Normal morph	71	0	3	96	3	>4%
Abnormal	29	100	97	4	97	NA
Head	NA	67	100	84.3	60.4	NA
Body	NA	40	63.9	78.1	93.8	NA
Tail	NA	23	42.2	8.3	19.5	NA

Table 2

Although sperms with abnormal morphology have been used for intracytoplasmic sperm injection (ICSI) successfully, several studies show that high rates of disomy and diploidy are related to severe teratozoospermia and therefore ICSI is not recommended in these cases[(1)]. A study performed by Kahraman in 2004 with macrocephalic spermatozoa showed that the quality of spermatozoa is indeed correlated to fertilisation and consequently with embryo development. It is proposed that a sperm-derived oocyte activation factor in abnormal spermatozoa may lead to an abortive oocyte activation and subsequently failure of pronuclear development[(1)]. Also, the configuration of the centrosome and aster formation which is critical in the development of the zygote may be defective in morphologically abnormal sperm[(1)]. Increased incidence of chromosomal abnormalities correlates with decreased semen quality as well as decreased pregnancy outcomes, and though there is not a standardized technique in sperm selection to discard chromosomal abnormalities and preserve the function for ICSI[(2)], sperm with morphological abnormalities should be discarded. However, other studies suggest that oligospermia and/or teratozoospermia do not appear to be associated with increased embryo aneuploidy but do correlate with poorer fertilization rates and embryo implantation[(3)]. Absolute teratozoospermia associated with sperm chromosomal abnormalities is well documented to be associated to a high rate of chromosomal abnormalities on preimplantation embryos, cycle cancellations, abortion as well as low fertilization, implantation and clinical pregnancy rates; though other forms of sperm chromosomal abnormalities and assisted reproduction failure should be studied further and though the selection of normal sperm for ICSI does not eliminate the chance of chromosomal abnormality in the infertile man[(2)], it does reduce the risk in a male with previous fertility proven, as our patient. Severe and/or absolute teratozoospermia in recent studies has been linked to

a three-fold and up to a 4.4 fold higher rate of sex aneuploidies in their embryos[4, 5]. Whereas there is controversy if, in fact, abnormal morphology is related to increased aneuploidy rate, some studies indicate that sperm morphology does not reflect chromosomal endowment and that ICSI is a suitable choice even if there is not even one normal sperm[4]; other studies have shown that patients with severe teratozoospermia (normal forms <10%) have a significantly higher aneuploidy rate in comparison with patients with a lower degree of teratozoospermia[6] as well as a higher risk of diploidy and polyploidy (Rodrigo). There is also a correlation between oligospermia and higher rates of teratozoospermia, as a checkpoint in meiosis leads to an arrest of abnormal cells[4,5]. The incidence of males with abnormal sperm morphology and increased rate of aneuploidies detected by FISH in sperm is higher in nonobstructive causes[5].

Patients with oligoasthenoteratozoospermia have synaptic chromosomal anomalies that are restricted to the germ cell line up to 26.7%, and between 8-12% of abortions with trisomies 13, 18, and 21 are linked to paternal origin[1,5] revealing the important role of sperm morphology to the proper development of the zygote. In the study conducted by Kahraman in 2006, no benefits from PGD were observed in the group of patients with zero normal morphology group[2] which gave the rationale of this paper: if the couple agreed to have ovum donation, sperm donation should have been offered as well, considering that with a high degree of abnormal sperm in the male, the chance of having aneuploid embryos was high, and the investment in the medical process was not worthy. It is important to underline that patients with severe teratozoospermia such as the male patient in this case who undergo ICSI, can display a higher rate of sex chromosome aneuploidies in their embryos, in comparison to moderate teratozoospermia[4]. Besides, the need for adequate diagnosis and treatment in the male should be considered, as some of the causes may be reversible. It is important that medical staff consider the well-being and economy of the couple regarding their decisions and health issues that may be not necessarily related to the reason for consultation. Directive consultation and adequate counselling are two different things and our role is to guide the patient in all possible outcomes that may be related to their health. The main objective of PGD is to diminish the probability of an early miscarriage (due to chromosomal abnormalities) and the emotional and economic impact that this event inflicts on the couples, therefore if there is by any chance augmented possibilities of not having a single euploid embryo due to previously known medical conditions, couples should be offered all available alternatives.

Male infertility has been a recurrent topic in recent studies, it is estimated that approximately 30 million men are infertile[4]. ICSI and PGD are powerful and helpful tools, ISCI reduces the requirements of semen quality samples, specifically for motility[4]; however we suggest in the cases of proven previous male fertility, as in this couple, reversible causes of teratozoospermia should be corrected or intended to correct before offering whichever assisted reproductive technology. In fact, recent studies show genomic abnormalities that lead to specific abnormal forms of the sperm, some of which are contraindications for ICSI, for example, AURKC mutations which lead to a higher risk of aneuploidy; or in all patients with globozoospermia due to an oocyte activation anomaly[7].

CONCLUSION

The approach to male fertility should be changed, though spermatobioscopy has been and is still a great tool, it is not a final answer, and more specific studies should be offered to patients to optimize treatment and reproductive strategies.

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Beckwith-Wiedemann Syndrome in a patient with full-term pregnancy, case report.

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ABSTRACT

Beckwith-Wiedemann Syndrome (BWS) is a rare congenital disorder caused by alterations to the IGF2 gene. Its incidence has increased with the use of assisted reproduction techniques. We present a case of a patient with BWS who experienced anaphylactic shock during delivery, likely related to oxytocin use. Multidisciplinary management is necessary in cases of prenatal diagnosis, with a focus on early correction of hypoglycemia and structural defects. BWS is associated with an increased risk of childhood cancer, and the use of assisted reproduction techniques may further increase this risk. Clinicians should be aware of the potential complications associated with BWS in pregnant patients and their newborns.

KEYWORDS: Beckwith-Wiedemann-Syndrome, pregnancy, anaphylaxis.

MANUSCRIPT

Introduction

Beckwith-Wiedemann Syndrome (BWS) is a multisystem congenital disorder characterized by excessive growth, hypoglycemia, and macroglossia. Its incidence is estimated at around 1 in every 10,000-13,700 cases births[1], with autosomal dominant inheritance, reduced penetrance, and variable expressivity. The syndrome is caused by various alterations to the IGF2 gene in the 11p15.5 region[2], which leads to heterogeneity in clinical presentation, mainly associated with growth disorders and alterations in insulin type 2 due to gene coding sharing.

Given the wide range of implications of these genes, various criteria have been cited to guide clinical diagnosis, including: Major Criteria: abdominal wall defect, macroglossia, macrostomia, embryonal tumors,

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malformations in the auricular pavilion, visceromegaly, hemi-hyperplasia, renal and ureteral anomalies, cleft palate; Minor Criteria: prematurity, neonatal hypoglycemia, flammeus nevus on the glabella, typical facies, placentomegaly, polyhydramnios, cardiomegaly, rectus diastasis, hemi-hyperplasia[1,3]. Diagnosis can also be made during the prenatal stage by performing amniocentesis, associated with cytogenetic, molecular, and biochemical studies, as well as obstetric ultrasound.

The association of BWS with childhood cancer is estimated to be between 4% and 21%, mainly embryonic tumors such as Wilms tumor (52%), hepatoblastoma (14%), neuroblastoma (10%), adrenocortical carcinoma, pheochromocytomas, and rhabdomyosarcomas[2,3]. However, the heterogeneity in its presentation has been understood based on the alterations that generally occur, with four main expression mechanisms being hypermethylation in IC1 (H19/IGF1:IG-DMR), hypomethylation in IC2 (KCNQ10T1:TSS-DMR), paternal uniparental disomy, and alterations in CDKN1C[4,5,6]. The presence of these mechanisms is related to a higher morbidity and mortality rate in infants due to differences in medical interventions for their proper management.

During recent years, with the growth of assisted reproduction techniques, an increase in cases of BWS has been observed, leading to the study of the association of these techniques. For instance, Vermeiden and Bernardus conducted a review to study the association between BWS and assisted reproduction techniques in eight epidemiological studies, finding a higher risk of developing BWS than in the general population, with a relative risk of 5.2 (95% confidence interval 1.6-7.4)[5]. Similarly, Mussa and Molinatto correlated the prevalence of BWS between the general population and the assisted reproduction registry in Piemonte, Italy, finding a higher incidence of BWS associated with assisted reproduction techniques, with a relative risk of 10.7 (95% confidence interval 4.7-24.2)[6].

In cases of prenatal diagnosis of BWS, a multidisciplinary management of the newborn should be carried out, focusing on properly guiding parents about possible risks and complications, performing complementary serological, molecular, and imaging studies, mainly focused on the early correction of hypoglycemia that immediately endangers the newborn's life, as well as managing structural defects related to the individual presentation of the disease[7].

Case presentation

A clinical case is presented of a patient diagnosed with BWS and a full-term pregnancy who experienced anaphylactic shock probably secondary to the use of oxytocin.

A 20-year-old primigravida presented with spontaneous membrane rupture three hours prior to admission and irregular uterine activity.

Past medical history: diagnosed with SBW at birth, with multiple corrective interventions associated with pathologies, such as correction of omphalocele (at birth), right mastectomy (at 10 years), right femoral reduction (at 12 years), and placement of a right breast expander (at 18 years). No allergies were reported.

Gynecological history: menarche at 9 years, menstrual cycle of 28x5, first sexual intercourse at 19 years, Papanicolaou smear 1, last menstrual period on January 10, 2021, prenatal care: 7 visits to the health center, no significant past medical history or infections during pregnancy.

The pregnancy was interrupted via abdominal delivery due to cephalopelvic disproportion attributable to the maternal pelvis, with a Kerr-type cesarean section using the Misgav Ladash technique, resulting in a female newborn, weighing 3,015 grams, measuring 48 cm in length, Capurro gestational age of 40 weeks, Apgar score of 8-9, and 300 ml blood loss. During the surgical procedure, the patient presented with hypotension, tachycardia, and a skin rash, and an echocardiogram and pulmonary ultrasound were performed during the surgery, which did not show any pathological findings. The anaphylactic reaction was managed with fluid therapy, norepinephrine, and hydrocortisone, which resulted in improvement, and was presumed to be related to the use of prophylactic intravenous oxytocin at a dose of 10 IU.

Discussion

SBW is characterized by multiple congenital defects related to morphological and structural alterations in the patient. While it does not directly affect fertility, it is directly associated with mortality in patients who present the syndrome and is also related to multiple endocrinological and oncological alterations. In addition, it can present with abdominal wall defects that can complicate term pregnancies.

CONCLUSION

SBW can occur in newborns through autosomal dominant inheritance in 15% of cases, with 85% being idiopathic. Monitoring of children of affected mothers should begin with detailed prenatal ultrasound to detect structural abnormalities. In adult patients, the endocrine system is affected due to a deficiency of hypothalamic hormones, as well as an increase in prolactin levels with a deficient response of luteinizing hormone, affecting fertility. Therefore, pregnancy is infrequent in these patients. It is essential to monitor

hearing loss every 2-3 years, the renal system with a renal ultrasound and urinary calcium every 3-5 years, and the cardiovascular system with echocardiography every 3-5 years[5].

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