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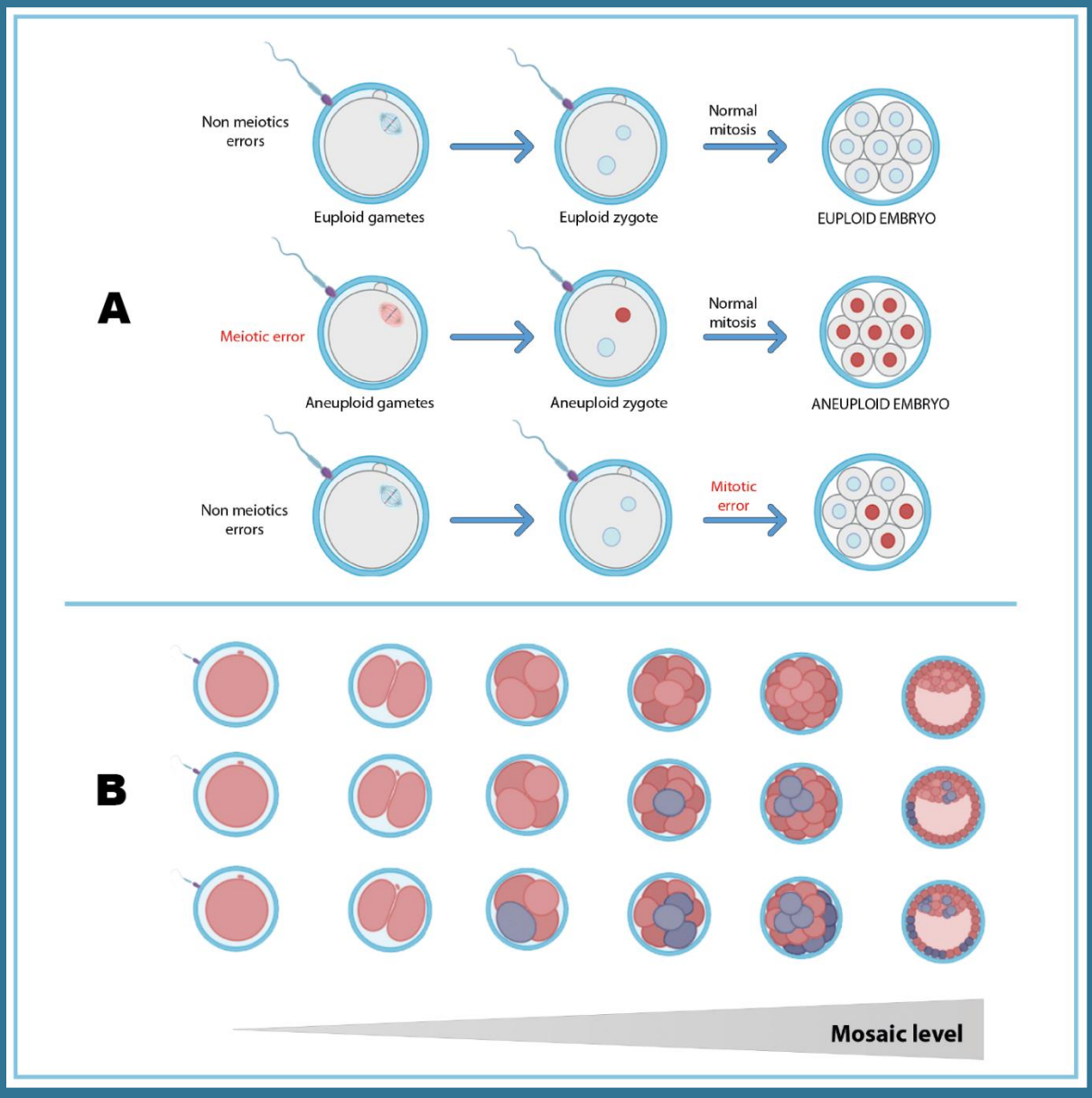

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The Journal of Reproduction

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Editorial

Asch-Schuff Ricardo Héctor[1], 0000-0001-5743-7121.

Greetings to our dedicated readers of The Journal of Reproduction, we are thrilled to welcome you once again to our latest issue, brimming with a diverse array of captivating articles. In this edition, we present a compelling assortment of up-to-the-minute reviews, groundbreaking research studies, and particularly noteworthy case reports that hold significant relevance for clinicians.

The enduring growth in both readership and submissions underscores the escalating interest in our journal as a platform for disseminating scientific insights and updates from pharmaceutical and assisted reproductive technology laboratories across the globe.

As promised, we are on track to deliver a special edition exclusively centered around the Endometrium before the year's end. Esteemed experts from around the world have enthusiastically contributed their cutting-edge research, promising to make this forthcoming issue an exhilarating compilation and a definitive source for those eager to stay abreast of the latest advancements in Endometrial studies.

We extend our heartfelt gratitude for your unwavering support, which remains instrumental in propelling our journal's continued success.

We trust that the contents of the current issue will both captivate and enrich your understanding.

Thank you for your ongoing commitment.

Warm regards,

Asch-Schuff Ricardo Héctor
Editor in Chief

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Embryonic mosaicism: What does it mean clinically?

Francisco Leocata Nieto [1], 0009-0001-2628-2267.

ABSTRACT

Embryonic mosaicism continues to represent a complex phenomenon that is considered by many authors as a limiting factor in the interpretation of PGT-A cycles results.

A mosaic embryo is characterized by the presence of cell populations with at least two different karyotypes. There are controversial aspects related to embryonic mosaicism, such as the great variability in the incidence reported between laboratories and clinics, which in turn is related to the existence of false positives during the diagnosis. These false positives can also affect the results after the transfer of these embryos. Numerous publications have analyzed the results of transferring this type of embryo. The majority agrees that although worse clinical results are achieved that, when transferring a euploid embryo, it is possible to achieve an evolutionary pregnancy with a healthy birth. The objective of this article is to provide tools that allow the professional to advise those patients who perform a PGT-A cycle about this conflictive aspect of it.

KEYWORDS: PGT-A; mosacism; mosaic embryo transfer; embryonic self-correction.

MANUSCRIPT

Introduction

Human reproduction constitutes a highly inefficient and selective phenomenon. Currently, even in optimal circumstances, the highest probability of achieving pregnancy is estimated at around 30-40 % per ovulatory cycle(1). The main cause of this inefficiency is due to the large proportion of generated aneuploid embryos, characteristic that correlates with clinical phenotypes, such as infertility and spontaneous abortion. This high prevalence of aneuploidies during pre-implantation also constitutes an important factor that contributes to the failure of assisted reproduction treatment (ART).

The preimplantation genetic test for chromosomal aneuploidies (PGT-A) has been transformed into an increasingly common practice in ART. PGT-A has widely shown its usefulness in advanced maternal age patients, implantation failure, recurring abortion, and previous pregnancies with chromosomopathies(2).

Although embryonic mosaicism has been known for decades, the greater precision and sensitivity of Next Generation Sequencing techniques (NGS) has provided the opportunity to identify more clearly an intermediate number of copies for a single chromosome(3), thus being able to detect a euploid-

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aneuploid mosaicism with greater frequency. It is currently considered that NGS can identify chromosomal abnormalities that are present in at least 20% of the cells (1 in 5)[5].

Embryonic mosaicism continues to represent a complex phenomenon that is considered by many authors as a limiting factor in the interpretation of PGT-A cycles results. In a mosaic embryo coexist at least 2 cell lines with different karyotypes, being able to coexist euploid and aneuploid cells or coexist only aneuploid cells with different anomalies.

Origin of mosaic embryos

While completely aneuploid embryos originate from an aneuploid zygote, derivative from one or both aneuploid gametes, mosaic embryos rise from an euploid zygote that suffers some abnormal mitosis (**Figure 1A**). The earlier this error occurred, the aneuploidy can spread to a greater number of cells, giving rise to embryos with a high percentage of abnormal cells (high-level mosaicism; **Figure 1B**). The occurrence of these mitotic errors could be due to the characteristics of these first divisions: the use of components and materials of oocyte origin (prior to the activation of the embryonic genome), the presence of more permissive cell cycle control mechanisms, etc.[6][7]. Recently, some publications have shown that the first mitotic division is highly susceptible to errors[6], suggesting it may be responsible for the early appearance of mosaicism.

There are several cellular mechanisms that can cause errors in chromosomal segregation during embryonic divisions, mainly those that result in bad segregations of sister chromatids. Within them, anaphase lag is considered the major cause of mosaic embryos. This chromosome lagging, together with the possibility of abnormal tripolar spindle formation that result in a massive loss of chromosomes ("chaotic" mosaicism), have been described as the main errors during the first mitotic division[6].

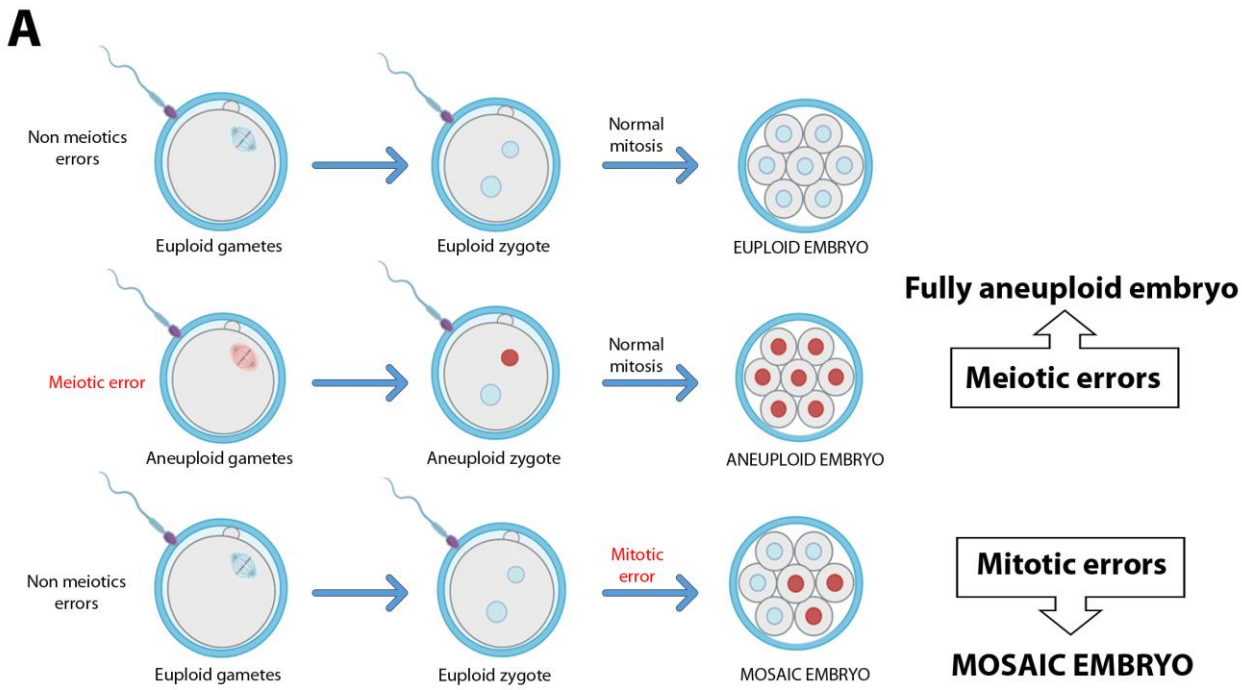

Figure 1. A. While euploid embryos originate from aneuploid zygotes, mosaic embryos come from euploid zygotos that suffer errors during mitotic divisions during the early stages of preimplantation development.

Factors influencing the generation of mosaic embryos

Few factors have been related to promoting embryonic mosaicism. Several publications have shown a greater tendency to produce mosaic embryos in couples with male factor, being even higher in the case of severe male factors and testicular sperm[9],[10],[11]. Any disorder of the sperm centrosome can theoretically produce mosaicism in the embryo. Sperm aster formation has been shown to be delayed in infertile males compared to fertile male controls. This could cause delayed syngamy and subsequent cleavage, and possibly induce aneuploidy and mosaicism. On the other hand, cellular stress factors such as variations in pH, osmolality and temperature

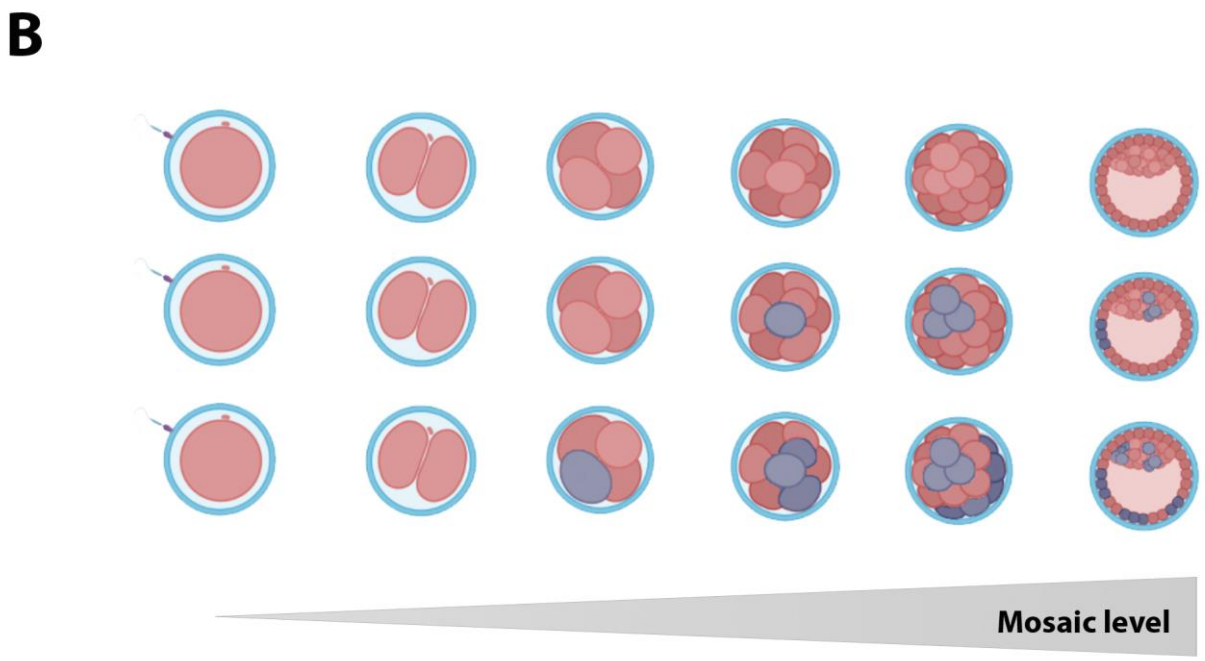

Figure 1.B. The earlier the mitotic error occurs, the greater the number of aneuploid cells present in the embryo.

can negatively impact mitotic divisions, affecting correct chromosome segregation[12].

While the incidence of meiotic errors and embryonic aneuploidies is highly related to maternal age, mosaicism appears to be unrelated to either maternal or paternal age. However, there are some publications that suggest a slight decrease in the rates of embryonic mosaicism in patients older than 37 years[13], [14].

Incidence of embryo mosaicism

Determining the incidence of embryonic mosaicism is a complex issue since its frequency varies considerably between clinics and analysis laboratories, depending on the detection technique, cells number and chromosomes analyzed, cells origin and thresholds established to define the levels of mosaicism (see Detection of mosaic embryos section). Instead, and as mentioned above, there are also certain biological factors related to culture conditions that can influence mosaicism levels.

According to the PGD International Society (PGDIS), the incidence of mosaicism reported at the blastocyst stage and using NGS methods varies between clinics, from 2% to 40%, although most clinics report levels between 5% and 15%[15]. ASRM recognizes levels of mosaicism from 3% to 20% depending on the NGS platform and the analysis parameters used[16]. CooperGenomis reports on its website a global incidence of 13.7% on more than 10,000 embryos analyzed[17]. Mosaicism levels in pre-compaction embryos are much higher, clearly reflecting that the presence of a certain percentage of aneuploid cells can compromise embryonic development. Among the wide variety of strategies that can be used, the analysis of multifocal biopsies (usually one ICM biopsy and 4 biopsies from different areas of the TE) represents the most credible approach to corroborate the chromosome mosaicism, although clearly not be of clinical utility. Popovic et al., using this technique, determined a mosaicism level of 37% in the studied population. Similarly, recently, Ren et al[18], using single cell sequencing, verified the presence of mosaicism in more than 60% of the analyzed embryos. As can be seen, there is great variability between the different laboratories, depending on the characteristics of the patients and the culture conditions of each place, but as will be seen later, it is mainly due to technical and methodological issues, related to diagnostic techniques.

In addition to this variability in the incidence of mosaicism, another factor that attracts a lot of attention is the great difference between the average levels of mosaic embryos detected and the values of mosaicism found in the products of conception (placenta/fetus). Placental mosacism has been reported in around 2% of the samples studied (similar values between natural conceptions and by in vitro fertilization[19]), and of these cases, the presence of fetus mosacism could be verified in only approximately 13%.

Mosaic embryo transfer

As initially mentioned, the use of NGS in PGT-A cycles was characterized by a significant increase in the in mosaic embryos reported, generating a great uncertainty regarding the prevalence of mosaicism in human blastocysts, its biological importance, and above all, the destination that should be given to these embryos. The decision to transfer these embryos contemplates not only that the mosaicism found in TE becomes a confined placental mosaicism, with the risks that this entails (intrauterine growth restriction, placental insufficiency, etc.), but also that it may be present also in the fetus, compromising its health. Chromosomal mosaicism has been related to the presence of various diseases such as Alzheimer's and frontotemporal dementia[20].

In 2015, Greco et al. were the first to report the clinical results after transferring mosaic embryos[21]. Currently, there are numerous publications that detail the results obtained after this kind of practice. In the publication by Treff et al.[22], a table is included detailing 25 highly relevant studies, in which the results obtained after transferring mosaic embryos are shown. Within these studies, the success rate is highly variable, depending on the number of embryos transferred and the goals set in each work (pregnancy rate, live birth, etc.). In this table we can also verify the birth of a single baby carrying the mosaicism initially detected in the embryo[23], after transferring 2759 mosaic embryos. After a pregnancy without any anomaly, a healthy baby was born at week 37, with no apparent phenotypic abnormalities, but with a peripheral blood karyotype showing chromosome 2 monosomy in 2% of cells studied. Beyond the characteristics of this specific case, the fact that it is the only pregnancy carrying a mosaicism among the 2759 transferred embryos represents a percentage of affected pregnancies well below the average value found even for embryo transfers without genetic analysis (0.04% (1/2759) versus 2%). This conclusion leads to doubts about the power of PGT-A to effectively detect mosaic embryos.

Among the studies listed in Treff's table, Viotti et al. reported the results of a multicenter study in which 1000 mosaic embryos were transferred[24]. The authors reported lower clinical results than euploid embryos (implantation rates, ongoing pregnancy and live birth, miscarriage) especially if embryos with mosaicisms involving complete chromosomes rather than segmental abnormalities are transferred. The results were even worse against high level mosaicism and against abnormalities involving multiple chromosomes. Despite this, the general population of mosaic embryos presented an implantation and evolutionary pregnancy of 46.5% and 37.0%, respectively. This allows us to conclude that despite presenting inferior clinical results to euploid embryos, mosaic embryo transfer should be taken into account, given that in certain cases it can lead to the success of assisted fertilization procedures, and that not using them would be condemning the storage to potentially viable embryos, with some possibility of leading to the birth of a baby. It is also important to mention that in this study no mention is made about the phenotype of the babies born.

Like many other retrospective studies, the previous publication presents a great bias from the strictly scientific point of view: mosaic embryos are transferred only in the absence of euploid embryos, that is, in suboptimal cycles, even in cycles where the patients have already transferred normal embryos without positive results. In Capalbo's study, embryos that showed a low or moderate level of mosaicism were blindly informed as euploid without distinction of uniformly euploid embryos. In this way, embryos were selected to transfer only based on their morphology. Considering the three groups (euploid, low level mosaic and medium level mosaic), no significant difference was observed in clinical parameters (pregnancy rates, abortion and living births). In some miscarriage cases (4/52), the abortion material was cytogenetically evaluated, not being aneuploid. Babies born presented normal obstetric and neonatal parameters, and in cases where a peripheral blood karyotype was performed (38/386 of born babies), these were also normal[25].

These two articles, like many others, seem to indicate that the transfer of mosaic embryos (at least those that do not have very complex anomalies) would represent a relatively safe practice, despite presenting clinical results lower than euploid embryos. Recently, Viotti and col., compared pregnancies resulting from embryos classified as euploid or mosaic, finding that babies born of mosaic embryo transfers are like babies of euploid embryo transfers[26]. On this basis, It must be considered that some potentially viable blastocysts are clinically classified as inappropriate to be transferred, negatively interfering with the result of an assisted reproduction procedure, especially in patients with few or no normal embryo. In this way, the idea of achieving conception products (placenta and/or fetus) free of anomalies despite transferring mosaic embryos, leads us to rethink real capacity of the PGT-A in reliably detecting embryonic mosaicism. To explain this phenomenon, we can travel deferens roads. One of these pathways is based on the existence of the so-called **embryonic autocorrection** phenomenon, which is based on the ability of an embryo, of being able to "eliminate" aneuploid cells.

There are different proposed mechanisms for this autocorrection to occur, which are not exclusive to each other, but could be acting synergistically to achieve

greater correction. Embryonic mortality and clonal exhaustion models suggest that aneuploid cells do not survive and are lost during implementation. A study in mice was the first to show the progressive exhaustion of aneuploid cells and in providing direct evidence that apoptosis in ICM is a mechanism to eliminate aneuploid cells[27]. In this study, the authors induced aneuploidies in early mouse embryos using reversine, then creating 8-cell chimeras embryos mixing aneuploid and control cells control. Embryos were cultivated to the blastocyst stage, checking a decrease in the percentage of aneuploid cells as a function of development time, due to an increase in apoptosis in the ICM. No variations were observed in the number of abnormal cells in the TE. On the other hand, chimeras embryos were created with different proportions of euploid and aneuploid cells. They verified that, if there is a certain relationship between euploid and aneuploid cells, lethality previously observed with aneuploidy embryos could be avoided, being able to achieve an evolutionary pregnancy.

hypothesis[31], there are several publications that do not show a preferential distribution of aneuploid cells in the TE[32]

Finally, it is suggested that aneuploid cells can lead to diploid cells through chromosomes loss or gain (trisomic/monosomic rescue model; (**Figure 3**). Some authors give little credibility to this model; however, the real presence of uniparental disomy (UPD, a consequence of this model) in natural embryonic suggests that it can occur, although in a very low rate: in a study of 3401 embryos, finding a general frequency of 0.06%[33]. A corrected monosomy using this model would always result in UPD while trisomy correction would result in UPD a third of the time. However, correction events that do not result in uniparental homologous are also theoretically possible and can be underestimated since they are impossible to detect.

Despite the seriousness of the above publications (and of several not included) that show evidence of the existence of this mechanism of embryonic rescue, justify the results obtained by transferring thousands of

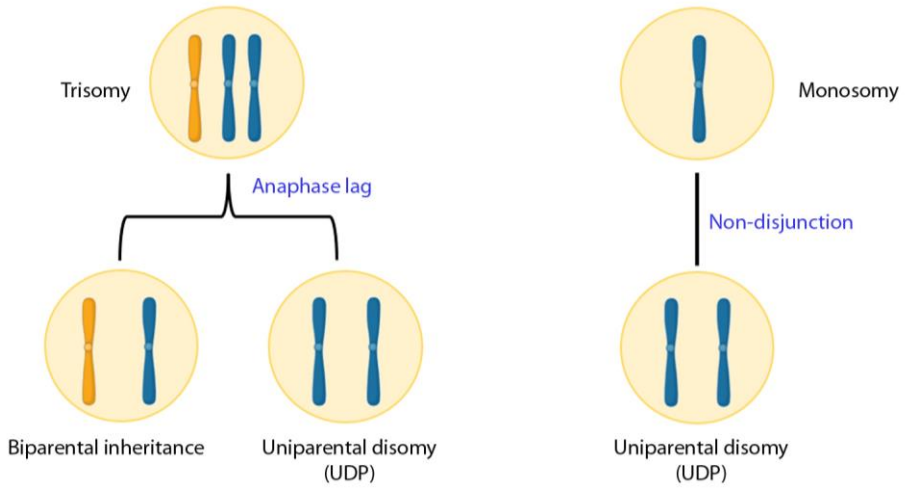

Figure 3. Aneuploid cells can lead to diploid cells through chromosomes loss or gain.

Another strategy for this selective elimination of abnormal cells is the exclusion of these from the developing embryo. It has been proven that the exclusion of aneuploid cells in the morula stage could also act as a potential self-correction mechanism[28], [29]. Many of excluded cells from euploid blastocysts are often aneuploids or with very fragmented DNA, and cells excluded from blastocysts with simple aneuploidies generally contain more complex aneuploidies and segmental aberrations[30].

Alternatively, the possibility that aneuploid cells are preferentially located in the TE has also been proposed. This hypothesis is controversial because, although there are studies that support this

mosaic embryos, in the absence of affected conception products, it would be overestimating the influence of these mechanisms. For this reason, several authors indicate that the absence of affected conception products is simply because these embryos were not effectively mosaic embryos, then being ***false positives***. This reasoning is developed in Nathan Treff's paper where the birth of a single baby affected after the transfer of more than 2500 mosaic embryos is mentioned, giving an incidence of affected births of approximately 0.04%[22], a value much less than 2% incidence detected in natural conceptions. This big difference cannot be explained solely by self-correction mechanisms.

The existence of these false positives should not be denied and is strongly related to the diagnosis of embryonic mosaicism. A systematic review where the results of the reanalysis of 289 embryos previously diagnosed as mosaic were linked to a single tea biopsy, could corroborate this result in only 42% of cases; The remaining embryos were diagnosed as euploids and aneuploidies (29% and 28% respectively)[35]. The transfer of these really aneuploid embryos, but considered mosaics, would be justifying (at least part) the lower clinical results, compared to the transfer of real euploid embryos.

These results clearly show how the correct diagnosis of a mosaic embryo remains a subject of continuous debate, mainly due to the doubts that are still presented about the capacity of the profiles with intermediate number of copies of a chromosome, to be able to correctly predict the presence of a true mosaic embryo.

Understanding the reason why an embryo can be diagnosed as mosaic, necessarily includes evaluating the factors involved and affecting its detection.

Mosaic embryo detection

From the point of view of the diagnosis, a mosaic embryo has an intermediate value of copies of a chromosome between monosomy/disomy or trisomy/disomy[15]. The diagnosis of mosaicism is highly complex and can be influenced by several methodological variables (**Table 1**).

We must start considering the analysis material, the TE biopsy. It is recommended that biopsies include between 5 and 10 integral cells. A lower number of cells could exclude euploid/aneuploid cells that would be defining mosaicism, while a larger number of cells could compromise embryo viability[36]. In addition, the simple fact that the diagnosis is based on the analysis of a single biopsy may be underestimating the phenomenon of embryonic mosaicism, since, as has been demonstrated in several publications, the distribution of abnormal cells is not uniform sowing within embryonic TE.

The state of biopsy cells is of great relevance, since the presence of cells with compromised integrity can lead to the loss or deterioration of the chromosomal material, conditioning the analysis. For this reason, different factors referring to the biopsy that must be taken to maintain the integrity of the cells and their chromosomal content must be considered. For example, the excessive use of the laser, the temperature and the time in the tubing, the washing conditions before the tubing, the shipping conditions to the molecular laboratory, are some of them.

The basic concept of a biopsy represents a limiting factor for the detection of mosaic embryos: on the one hand, biopsy can include only euploid cells (or aneuploids), leaving aside a population of aneuploid cells (euploids) located in another sector of the sector of the TE (**Figure 2A**), or may include cells with complementary aneuploidies that as a whole would complete the disomy; in both cases, embryonic mosaicism would be masked by a technical artifact (**Figure 2B**).

As is known, whole genome amplification (WGA) allows transforming the small amounts of DNA obtained by the embryonic biopsy, in quantities that can be analyzed by modern molecular techniques. However, WGA methods can lead to inefficient amplification, which leads to insufficient or excessive representation of some part of the genome[22]. The most modern WGA protocols, such as those based on MALBAC technology, tries to minimize these errors.

Biopsy-associated factors	NGS-associated factors
Biopsy technique	Amplification protocols (Whole genome amplification)
Biopsied cell quality	Platform specificity and sensitivity
Cell number	Thresholds or limits for diagnosis

Table 1

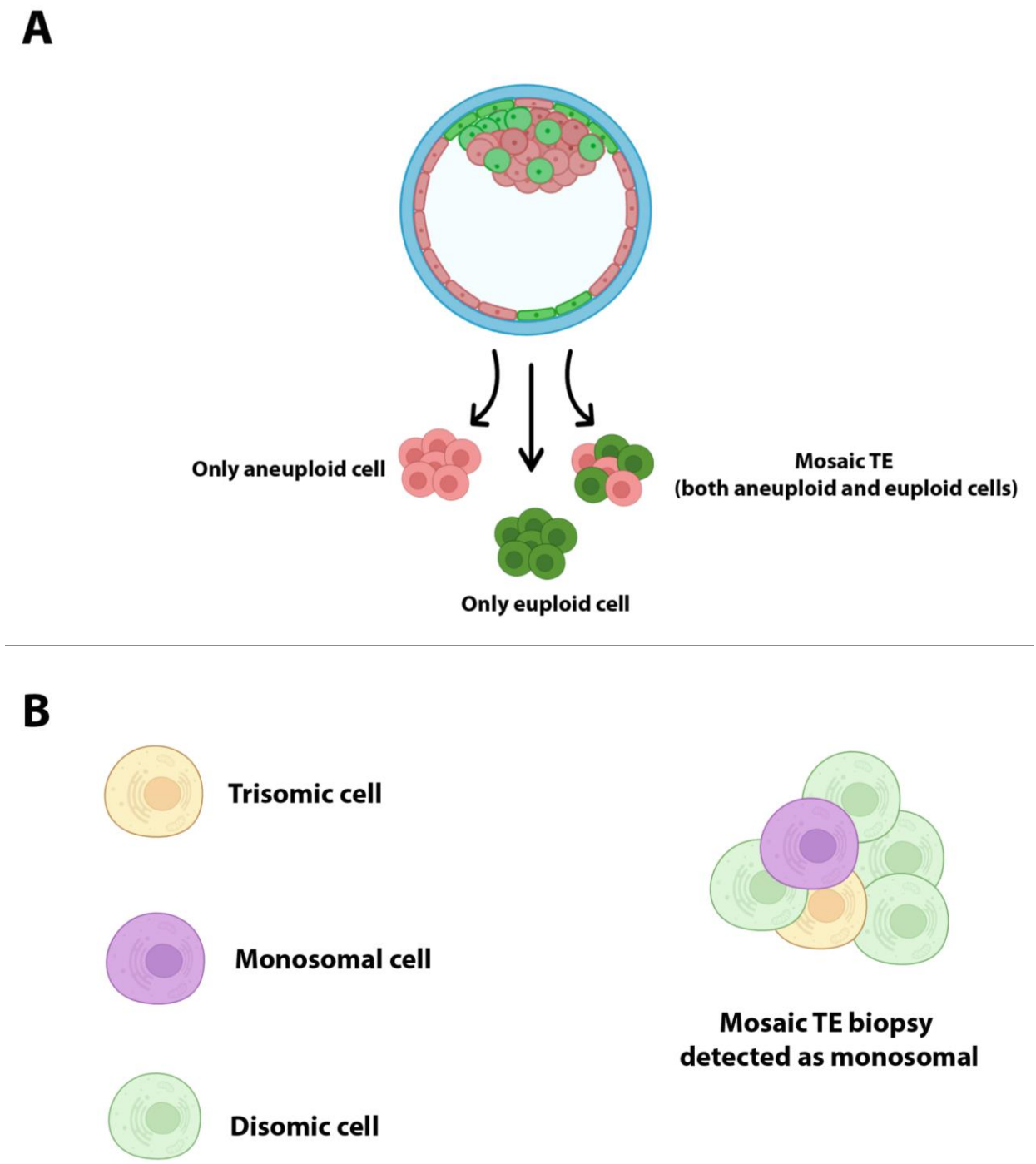

Figure 2. The characteristics of the biopsy of you can influence the result of the chromosomal diagnosis.

Another important factor to evaluate is the concept of "thresholds". Most laboratories use one of the following criteria to consider an embryo as mosaic and establish whether the embryo carries a "high" or "low" level mosaicism. Some laboratories consider an embryo as mosaic when TE biopsy evaluated contains between 30% -70% of aneuploid cells, outside those limits, the embryo is considered as euploid or aneuploid. In this way, it is also defined as an embryo with “low level” mosaicism when it has 30% -50% of aneuploid cells, while it is considered as a mosaic embryo of “high level” when biopsy contains 50%-70% of aneuploid cells. Other laboratories use a less strict criterion of 20%-80%, establishing low- and high-level mosaicism between 20%-40% and 40%-80%. The choice of one of these (or some other) thresholds, has direct influence on the diagnosis. Less strict limits (20%-80%) increase sensitivity to detect mosaic embryos but with decrease in detection specificity since it increases the percentage of false positives[37].

NGS platforms also have must be evaluated and validated to determine their ability to detect embryonic mosaicisms. Classically, platforms have been validated using artificial mixtures of euploid cells and aneuploids, a strategy that is far from what happens with a TE biopsy. The platforms must have a specificity that allows it to clearly distinguish the biological signals product of the detection of a certain sequence, of the technical noise of the equipment.

Although what is expressed until now is a brief description of the technical inconveniences that surround the diagnosis of embryonic mosaicism, they are enough to highlight the complexity of the detection of a mosaic embryo.

The need to optimize the diagnostic and results interpretation methods in order to have a reliable determination of embryonic mosaicism, is clear and accepted. In this regard, recently Buldo-Licciardi published a study that uses artificial intelligence (AI) for the correct interpretation of NGS results, with the aim of reducing human subjectivity in the interpretation of the results, improving the sensitivity and specificity of the diagnosis. Using this platform, a more precise diagnosis was achieved that positively impacted the clinical results[38].

Final considerations

From all the above we can affirm that:

1. The diagnosis of embryonic mosaicism constitutes a challenge of great complexity, characterized by the different methodological challenges, which must be considered to avoid falling into false positives.
2. The experience developed so far would seem to indicate that mosaic embryos transfer would be a relatively safe practice, since it does not correlate with a high probability of generating of conception affected.
3. The clinical results obtained when transferring mosaic embryos are more unfavorable than those obtained by transferring fully euploid embryos, especially as the complexity of the chromosomal abnormalities present increased.

Based on these statements, we would not have to question whether it is possible to transfer a mosaic embryo, but we should reflect on what mosaic embryo we can transfer. To collaborate with these decisions, various scientific societies have provided some guides in this regard[15], [16], [39]. While each statement has its own premises, they all agree in some characteristics in common:

- Mosaic embryos should be transferred only in the absence of available euploid embryos.
- Couples should take knowledge of everything that implies the transfer of these embryos and provide their consent.
- Each society has different guides to prioritize the transfer of a mosaic embryo over others, based on the chromosomes involved and the level of mosaicism, mainly.

In 2018, Grati and collaborators published a study on samples of chorionic villi and products of conception. The authors developed a practical guide that evaluates the real risk of transferring a mosaic embryo, evaluating the phenotypes detected in natural conceptions, and based on chromosomal anomalies[40].

There are many publications that support the decision to transfer mosaic embryos, evaluating the relevant risks, as well as the specific characteristics of embryo anomaly. This decision should be taken by health professionals with patients. A couple must know, before starting a cycle of PGT-A, all the advantages and limitations of this technique, including the possibility that some of their embryos be diagnosed as mosaic. They must be informed about the incidence of this phenomenon, the considerations regarding their diagnosis, and the possible risks they imply deciding transfer them. They must know that the transfer of embryos in mosaic is associated with lower implantation rates and a greater risk of spontaneous abortion, than when transferring euploid embryos. It is also important to mention that, although so far more than 100 births have been reported by transfer of embryos in mosaic, without abnormal phenotype, greater long -term studies are necessary to define their true security, given that almost all these babies do not reach 10 years of age.

The existence of large incidence of false positives in relation to the embryonic diagnosis is clearly affecting the decision related to the transfer of a true mosaic embryo. On the other hand, the results obtained by multifocal biopsies and individual cell sequencing[1], [18], as well as studies that indicate the large rate of mitotic errors during the first preimplantation divisions[6], seem to indicate that real mosaicism rate is superior even to the estimated. However, there would be different biological mechanisms that could ensure the development of a healthy pregnancy, eliminating abnormal cells, if the number of normal cells is sufficient[28].

In normal tissues, alterations of the number of copies of chromosomes that range between 1.1% and 10.6% are detected, without compromising its

functionality[41]. The appearance of an aneuploidy induces complex cellular responses that affect cell destination. Convincing evidence of cells in culture suggests that the appearance of aneuploidies is associated with the activation of roads that mediate cell stress, which can reduce the proliferative capacity of these cells. When these cells can counteract these effects, they have great risks of transforming into tumor cells[42]. In addition to cancer, the appearance of aneuploid cells has been linked to other physiological processes, such as development and aging. In fact, in some tissues, such as brain and liver, the appearance of aneuploid cells seems beneficial[43]. From this point of view, the existence of a few aneuploid cells within the approximately 200 cells that form a blastocyst (as in the case of low -level mosaicisms), may not be compromising the healthy course of an eventual pregnancy and birth.

We have advanced a lot in the understanding of embryonic mosaicism, and perhaps, over time we realize that we have been overestimated this phenomenon.

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CONFLICT OF INTEREST

The author declares he has no conflict of interest.

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Participation of vitamin D endocrine system in human fertility.

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ABSTRACT

Interest is growing in the knowledge of the mechanisms of action of vitamin D in very different areas of pathology. In recent years, very different actions of vitamin D have been discovered. Far from being a simple vitamin, it is a hormonal complex that acts in very different parts of the body equipped with hormonal receptors for vitamin D. It controls more than 900 different genes making it. It represents up to 3% of the total human genome. There is a deficit in levels of 25 (OH)D in populations around the world, including young couples affected by fertility problems. In the field of infertility, it has shown beneficial actions on the evolution of polycystic ovary syndrome. It is capable of decreasing the growth of uterine fibroids and correlates well with anti-Mullerian hormone. It has also been shown to improve oocyte quality and the embryo implantation rate in assisted reproduction: Its adequate levels in pregnancy reduce the risk of gestational diabetes and improve perinatal outcomes: The objective of this manuscript is to review the most important aspects that relate to the endocrine system. of vitamin D with fertility and pregnancy.

KEYWORDS: Vitamin D. Fertility. Infertility. Cholecalciferol. Calcifediol.

INTRODUCTION

In recent times, we have been witnessing a true bibliographic boom of publications on vitamin D (VD) that exceeded 100,000 indexed according to new data[1]. Since 2021 alone, 447 meta-analyses have been published on various topics in relation to VD. Differentiating the two basic drug forms of VD endocrine system (VDES), cholecalciferol and calcifediol, since 1945 the investigations and the data do not stop growing as shown in **Table 1**.

Product								
	1945-2020	2010.20	2020-23	10 years	5 years	1 year	5 years	1 year
Cholecalciferol	27.378	7.559	2.688	1.351	685	55	74	22
Calcifediol	4.040	1.641	694	226	104	18	29	13

Table 1. Constant increase in the number of VDES publications, differentiating cholecalciferol and calcifediol, barely marketed since 1943* (own elaboration from citation[1]).

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The presence of VD receptor (VDR) in many different tissues related to fertility (breast, epididymis, hair follicle, embryonic muscle, ovary, placenta, prostate, testis, uterus, yolk sac..., among others)[23] justifies that researchers have devoted special interest to the relationships between VDES and the different disorders that cause infertility. It seems evident today that VDES has different actions in different age groups, and in young women and men it can have influence on fertility and reproductive performance[4].

It is amply justified to affirm that it really is a hormone[2,5], considering the classical criteria of the hormone concept and its mechanisms of action[3] with an extraordinarily complex secretion and metabolism that involves the skin, the liver and the kidney until obtaining the metabolically active product[4], so we will no longer insist on its name as VDES.

The objective of this manuscript is to review the relationship between different levels of 1,25(OH)2D as the best determinant of its actions[6] and the main causes that negatively influence the fertility; In the same way and secondarily, we will briefly review whether there is justification for certain therapeutic regimens of Vitamin D in the different fertility diagnoses and in pregnancies, based on the results of gestational complications and perinatal results.

INFLUENCE OF VDES ON FERTILITY

1.- Hypovitaminosis D in infertility patients.

The specialized literature generally agrees that 25(OH)D levels are low in different regions of the world[7], and this would even include healthy young people from subtropical regions of southern Europe (presumably aware of the importance of maintaining these levels due to their condition as medical students), as demonstrated in a study carried out in the Canary Islands[8]. It is therefore not strange to think of the possibility that the same finding could be observed among patients with fertility problems in relation to 25(OH)D levels.

A high rate of hypovitaminosis D has been observed in women of childbearing age. The current results of a well-designed study show that circulating 25(OH)D levels fluctuate seasonally in a cohort of women who seek medical help for partner fertility despite residing in the northern part of Italy, in a sunny region like southern Europe. Similarly, vitamin D (25(OH)D) status is associated with specific causes of infertility and the physical characteristics of everyone. The authors of the study that showed these findings conclude that the use of supplementation continues to be a problem in this population and reproductive physicians should consider this aspect in their clinical practice[9].

2.- VDES and polycystic ovary syndrome (PCOS).

It is known that about 67 to 85% of women diagnosed with PCOS are deficient in 25(OH)D or calcifediol[10]. It is also known that VDES is involved in the pathogenic mechanisms of metabolic imbalances, the true pathophysiological motor of PCOS[11].

It has been argued that the metabolic syndrome that accompanies PCOS is at the crossroads of the metabolic disturbance that leads to anovulation; this being the case, maintaining adequate levels of 25(OH)D would be the basis for improving results with this type of patient. A meta-analysis has recently been published that reviews whether vitamin D supplementation in this group of patients would be justified[12]. Well, the authors evaluated a total of 11 studies (among the more than 446 previously selected) that were methodologically well designed for the stated objective. They conclude that the evidence from the randomized clinical trials (RCTs) reviewed suggests that supplementation of PCOS patients with continuous low doses of vitamin D (<4000 IU/day) or supplementation with vitamin D as a co-supplement may improve sensitivity to insulin in terms of fasting glucose concentration (vitamin D supplementation in combination with other micronutrients) and significantly improve the HOMA-IR index (Homeostasis Model Assessment and Insulin resistance) or homeostatic model to assess insulin resistance[12], a one of the most clinically proven tools to assess insulin resistance and pancreatic beta cell function, a true workhorse of the future for women affected by PCOS[13].

3.- Relations between VDES and uterine myomatosis (UM).

It is difficult to define an association between infertility and UM due to the heterogeneity of fibroids in terms of location, size, and number, as well as the different prevalence rates observed among different patient populations[14]. Notwithstanding this, VDES suppresses cell proliferation and cell growth, causing a reduction in MU[15]; furthermore, it acts as a suppressor of transforming growth factor-beta (TGF-β), which is involved in the development and progression of MU16. In fact, sufficient serum 25(OH)D levels were associated with a 32% reduced risk of UM compared with those with insufficient 25(OH)D [OR = 0.68, CI (0.48–0. 96)], regardless of the ethnicity studied[17,18].

From a therapeutic point of view, vitamin D supplementation after 12 months restored serum 25(OH)D levels in women with hypovitaminosis D and reduced MU growth, suggesting that it is an effective therapeutic strategy to prevent vitamin D deficiency. surgical intervention in small fibroids (<5 cm in diameter)[19]. Even with only 10 weeks of vitamin D

supplementation (50,000 IU), serum 25(OH)D levels were significantly higher in patients with 25(OH)D deficiency (36.08 ng/mL vs 16.25 ng/ml) p < 0.001) and the MUs decreased significantly in size[20].

The scientific evidence is not entirely conclusive. No statistically significant decrease in fibroid volume was observed in the experimental group [mean difference (MD): -0.71, 95% confidence interval (CI): -0.1 to 1.53, p = 0.085], in a recent RCT, but its additional growth was prevented[21]. To overcome the major limitations of previously published studies, which included only a small number of subjects, an ongoing open-label RCT involving more than 2,000 Chinese individuals is currently evaluating the efficacy of vitamin D supplementation in reducing the incidence of UM in women of reproductive age[22]; no results have been reported to date[23].

Despite the lack of a clear consensus, the treatment with calcifediol or cholecalciferol could be a potentially economical treatment for the prevention of further growth of UM and the treatment of uterine fibroids[24,25].

4.- Status of anti-Mullerian hormone (AMH) and calcifediol.

AMH is a member of the transforming growth factor beta (TGF-β) superfamily. It is a homodimer disulfide-linked glycoprotein with a molecular weight of 140 kDa, whose gene is located on the short arm of chromosome 19 in humans, band 19p 13.3[26]. A significant advantage of serum AMH is its low intracycle and intercycle variability, since it is produced from small antral follicles, which are independent of gonadotropins. Its strong correlation with the number of follicles and its high negative predictive value for premature ovarian failure (POI) make it an attractive tool in the study of infertility. It also helps in the individualization of assisted reproductive techniques (ART) protocols, thus minimizing iatrogenic effects and the total cost of the procedure.

In this context, women with 25(OH)D <30 ng/mL in follicular fluid have increased mRNA expression of AMH receptor type II in granulosa cells of small follicles, suggesting an important role for 25(OH)D in the expression and signaling of the AMH gene[27]. Interestingly, after controlling for seasonal fluctuations, a negative linear correlation was found between AMH levels and 25(OH)D levels only up to ca. 30ng/ml (p= 0.06). Beyond this value, there was no statistically significant relationship (p = 0.50)[28].

In another similar study, the authors found a positive correlation between serum 25(OH)D levels and AMH levels in late reproductive age (>40 years) (regression slope = +0.011; p= 0.028). However, in women <35 years of age, and after adjustment for covariates, an insignificant correlation was observed between 25(OH)D and AMH (r 2 = −0.0086; p = 0.054)[29]. However, again, the evidence is not always consistent; in women trying to conceive spontaneously, 25(OH)D levels did not correlate with AMH, but there was a tendency to associate insufficient 25(OH)D levels <30 ng/ml with low AMH (<0.7 ng/ ml) [OR 1.8, CI (0.9–4)][30]. Similarly, another cross-sectional study that included infertile women with a high prevalence of decreased ovarian reserve confirmed the lack of association between serum 25(OH)D levels (<20 ng/mL vs. ≥20 ng/mL) and AMH. (0.8 ± 3.0 ng/mL vs. 0.5 ± 1.6 ng/mL, p = 0.1761, respectively) after adjusting for age, BMI, and seasonal fluctuations[31]. Finally, in the same sense and in a mainly caucasian population without 25(OH)D deficiency (69.3% ≥20 ng/ml), no correlation was found between vit D and AMH[32].

5.- VDES and assisted reproductive techniques (ART).

In this specific area of knowledge, the agreement seems more unanimous whenever the evidence is uniform. In fact, in a systematic review that correlated 25(OH)D levels and ART results[33], most of the studies reviewed reported a decrease in ART results in patients with vitamin D deficiency. Specifically, in 34 studies reviewed, only one showed unrelated results, two showed to be indifferent, and the remaining thirty two showed positive correlations[33]. Cost-benefit analysis suggested that vitamin D screening and supplementation prior to ART might be profitable, but, as usual, more evidence is needed. Given the absence of level I evidence regarding vitamin D status and ART outcomes, it is premature to fully support 25(OH)D screening and supplementation prior to ART, but because the low complexity of the measure and the indicated results we think the suggestion valid[33].

Another study compared prospectively the results of in vitro fertilization (IVF) in a group of 173 patients, of which only 78 had sufficient levels of 25(OH)D[34]. The two groups were epidemiologically homogeneous (sufficient vs. insufficient or deficient), pregnancy rates per cycle started were 52.5% vs 34.75 (p<0-001) and 54.7% vs 37.9% (p<001) per embryo transferred, respectively. These findings, the authors report, suggest that women with sufficient levels of vitamin D are significantly more likely to achieve a clinical pregnancy after IVF. Vitamin D supplementation, they argue, could provide an easy and cost-effective way to improve pregnancy rates, this warrants further investigation. Therefore, it may be beneficial to determine vitamin D status as part of routine infertility evaluation and before artificial reproductive treatment, especially in women with a higher BMI. This study was registered before its development and had international controls[35].

In recent years, the general trend behind the innumerable improvements in embryo culture techniques is the trend towards single embryo transfer. In this context, 25(OH)D levels have also been evaluated in IVF[36] cycles, confirming that vitamin D deficiency affects pregnancy rates in women who undergo a single blastocyst transfer. In this case, the group was much larger (239 patients against 129 but only discriminated by having the first <20ng/ml of 25(OH)D in plasma vs >20 in the other group). The clinical pregnancy rates from the same study (beyond the biochemicals) of 52% vs 67% in both groups were equally significant ($p<0.015$); but beyond that, the rates of "baby at home", the true objective of ART, were still equally different ($p<0.015$), (35% vs 48%, respectively).

We still do not know the precise mechanism by which VDES exerts these positive effects, which have been demonstrated in practically all the studies reviewed in this regard, regardless of whether they were retrospective or prospective, as the first author of this paper demonstrated in a previous review[25]. In this regard, the results of a study carried out more than a decade ago that correlated embryo quality with 25(OH)D levels measured in the follicular fluid obtained on the day of IVF oocyte retrieval can help us[37]. The authors started from the division of the patients between pregnant women after IVF (n=26) versus non-pregnant women (n=58); well, in addition to differing significantly by consuming fewer doses of gonadotropins in the stimulations in the former ($p<0.001$), the latter consumed fewer days of stimulation ($p<0.002$), all of which is perfectly logical when dealing with patients with better performance in IVF. The most important thing, in the same order of things, is that pregnant patients showed significantly higher intra-follicular 25(OH)D levels than non-pregnant patients (34.42 ng/ml vs 25.62, $p<0.013$) also receiving significantly higher number of embryos (2.56 vs 1.98, $p<0.011$). The differences were even greater for patients who were in the highest fertile of intra-follicular 25(OH)D with embryo implantation rates greater than 35% and pregnancy rates greater than 50% per attempt. The authors conclude that the findings that women with a higher level of 25(OH)D in their serum and follicular fluid are significantly more likely to achieve a clinical pregnancy after IVF and embryo transfer are novel.

In relation to the mechanisms that support the effect, they suggest a potential benefit of vitamin D supplementation in the success of treatment in infertile patients undergoing IVF and deserves further investigation, and deserves further investigation, as the first author of this paper concluded in his recent review on the topic[25], which would at least suggest generalizing the determination of 25(OH)D as part of the fertility study even before deciding whether the patient is a candidate for IVF or a different ART in order to achieve the desired pregnancy in the fewest number of attempts possible.

INFLUENCE OF VDES IN PREGNANCY

1.- Vitamin D and gestational evolution.

To the extent that SEVD is responsible, among other effects, for intestinal calcium (Ca++) absorption, the adaptive processes of calcium homeostasis in human pregnancy and lactation, compared to normal[38], are well known. and situations of loss of bone mass during pregnancy with diagnoses of established osteoporosis have even been described among especially predisposed patients who have suffered low-impact fractures due to high calcium consumption during this period.

In a similar way to what we have pointed out among infertility patients, the geographical location of the residence and the number of hours of sunlight exposure in the area do not in any case guarantee adequate levels of 25(OH)D in a group of pregnant women analyzed. Pérez López et al.[39] detected up to 63% of deficient or insufficient pregnant women in a group of pregnant women in Almería (an area with more than 3,500 hours of sunshine per year), making it irrelevant whether the women analyzed were Spanish or of foreign nationality, mostly North Africans in that region[39]. Moreover, the situation was not significantly different in the different seasons, so they concluded that living on the Mediterranean coast of Spain does not guarantee good 25(OH)D levels during pregnancy or in summer. The logistic regression analysis on the factors related to the low state of 25(OH)D < 20 ng/mL, were among others less striking, the BMI ≥25 (Odds ratio 0.48 IC 95% [0.28-0.84]) and being of Caucasian race (OR 0.18 95%CI [0.10-0.31])[39].

Almost twenty years ago, from experimental studies with KO mice genetically predisposed to developing diabetes mellitus (DM), the relationship between 25(OH)D levels and the possibility of developing DM throughout their lives[40] was known. In the same way in males as in females, those deficient in vitamin D at the beginning of life developed significantly more DM (66% vs 45% $p<0.01$ and 35% vs 15%, $p<0.005$, respectively for males and females), during its existence[40].

2.- VDES and gestational DM (GDM) development.

The extrapolation in humans of the findings observed in mice comes from the meta-analyses that try to correlate vitamin D and GDM. Some authors have described that RCT data remain limited, but are critical to understanding whether vitamin D supplementation,

beyond what is contained in routine prenatal vitamins, will prevent GDM or improve glucose tolerance in women with DMG[41].

Plasma markers of 25(OH)D status and insulin resistance (IR) during the 1st trimester and late pregnancy before and after daily oral supplementation of 200 IU, 2000 IU and 4000 IU demonstrated (with small groups of 35, 38 and 40 patients respectively for the three doses of cholecalciferol), which significantly improved not only the 25(OH)D levels before and after oral supplementation, but also the basal insulin levels, the HOMA-IR index, and glycemia. baseline and plasma Ca++ levels[42].

In the same sense, an observational cross-sectional study that included 160 pregnant women between 20-40 years of age, in the third trimester, demonstrated a statistically significant negative correlation (r = -0.245) between glycemic control and plasma 25(OH)D levels in the entire study population[43].

Up to 2014, two well-conducted meta-analyses reported evidence of an association between a low 25(OH)D level and increased odds of gestational diabetes mellitus (GDM). However, it is still unknown whether vitamin D deficiency contributes to the pathophysiology of GDM development. To our knowledge, none large randomized trial of multiple doses has been published. The only RCTs available were promising, but far from definitive, and RCTs were essential to prove a protective effect of optimal 25(OH)D status with respect to the development or management of GDM[41].

Later, in 2015, Chinese authors published a meta-analysis of observational studies, but with a good design and confirming two coincident findings[44]. Meta-analysis of 20 studies including 9,209 participants showed that women with 25(OH)D deficiency compared with control experienced an increased risk of developing GDM (OR = 1.53; 95% CI, 1.33, 1.75). But at the same time, they verified that the serum level of 25(OH)D was significantly lower in the participants with GDM than in the control (95% CI, -6.73, -3.14) p = 0.001. They conclude by stating that there is a consistent relationship between 25(OH)D deficiency and increased risk of GDM, and a significant decrease of 4.93 nmol/L (~12.5 ng/mL) in serum 25(OH)D in GDM participants. However, once again, well-designed RCTs with robust n are needed to determine the explicit effect of vitamin D supplementation in the prevention of GDM. Until then, one could consider screening women at risk of VD deficiency and supplementing them with vitamin D, as already noted in our consensus papers[6].

3.- VDES and perinatal results.

It is known that the interaction of VDES and pregnancy correlates with different VDR[2,3]. The 24,25(OH)2D3 (24,25 dihydroxy-cholecalciferol) synthesized by the placenta accumulates in bone and may be involved in the ossification of the fetal skeleton[45]. Through its much more complex non-genomic and genomic actions, vitamin D plays an important role in pregnancy, with emphasis on immune function and fetal ossification. It is known that there are main changes in the fetal-placental unit that condition the transfer of maternal calcium to the fetus[46]. All this conditions a whole series of changes in BMD (bone mineral density) of the ultradistal radius (measured by US - ultrasound), during pregnancy and postpartum[47], which cause loss of bone mass already in the 10th week of gestation that continues in the week 22nd and 34th when 92 pregnant women were compared to 75 non-pregnant women ($p<0.05$)[38], which would be at the base of the already mentioned possibility of even developing gestational osteoporosis[38, 46, 47].

A systematic review of 76 studies carried out a few years ago highlighted the unfortunate heterogeneity of the studies found and the need, once again, for adequate RCTs with a good design and sufficient n. The authors noted that at least at that time, the evidence base is insufficient to support definitive clinical recommendations regarding vitamin D supplementation in pregnancy. Although they found modest evidence to support a relationship between maternal 25(OH)D status and newborn weight (effect size 5.63 with 95% CI [1.11-10.16]), bone mass and serum calcium concentrations of the offspring, among the children of women treated with vitamin D during gestation (0.005 with CI 95% [0.02-0.07]), these findings were limited by their observational nature (in the data of birth weight and bone mass) or risk of bias and low quality (regarding calcium concentrations)[48].

The situation is probably universal; In tropical countries, vitamin D deficiencies have been repeatedly demonstrated among normal pregnant women49. Other evidence from well-designed studies suggests that vitamin D exposure during fetal development influences the newborn's immune system, which may contribute to protection against asthma-related outcomes, including infections, in the early years. of life[50]. This would be in perfect synchrony with the already known anti-inflammatory action of SEVD in terms of protection against infectious and viral diseases in particular[51,52]. Thinking that young or healthy people have sufficient levels of 25(OH)D just because they live in sunny areas, even with subtropical climates in southern Europe[8] or even tropical (almost 40% deficiency or insufficiency among women of reproductive age in Colombia)[53], it seems a profound error of presumption, based on the accumulated evidence.

GENERAL CONCLUSIONS

1. The presence of VDR in multiple tissues related to fertility and pregnancy allows to predict a determining role of VDES in human reproduction in general.

2. A situation of hypovitaminosis D has been repeatedly demonstrated among patients affected by infertility, even if their place of residence receives a sufficient level of solar radiation.

3. The finding of deficiency or insufficiency of 25(OH)D is common in patients with PCOS.

4. Likewise, 25(OH)D levels correlate inversely with the development of uterine fibroids and supplementation could be an adequate therapeutic alternative in selected cases.

5. Women with lower 25(OH)D levels tend to have lower AMH values in their fertility studies.

6. Patients undergoing IVF have better results in terms of pregnancy rates when their 25(OH)D levels are normal or supplemented before starting treatment.

7. While the exact mechanism is still unknown, VDES produces better results in embryo implantation rates when intra-follicular 25(OH)D levels are higher, even in cycles with single blastocyst transfer.

8. Extensive situations of hypovitaminosis D have been described among (young) pregnant women, including residents in the sunny south of Spain.

9. Pregnant women with lower levels of 25(OH)D have a higher risk of developing GDM and pregnant women with GDM have lower 25(OH)D levels than controls.

10. Adequate 25(OH)D levels allow predicting a better newborn weight and a better response from the immune system in general.

11. Although the accumulated evidence is limited and heterogeneous, it always points in the same direction of the improvement of fertility and pregnancy due to the action of VDES.

12. More RCTs with good design and large groups are needed to elucidate the mechanisms involved in these relationships, as well as confirm the limited scientific evidence to date.

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Reproduction and Anti-Obesity Medications: A Review of Current Evidence

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ABSTRACT

Obesity, typified by the disproportionate accumulation of body fat, constitutes a burgeoning global health dilemma with far-reaching impacts on numerous facets of human well-being, prominently inclusive of reproductive health. The pervasive effects of this worldwide health predicament are both direct and indirect, having a compelling influence on fertility, thus underlining the exigency for efficacious therapeutic interventions. FDA-approved anti-obesity pharmaceuticals, comprising metformin, orlistat, phentermine, and glucagon-like peptide-1 (GLP-1) receptor agonists such as liraglutide and semaglutide, have been substantiated to facilitate weight loss and enhance metabolic indices. However, despite their promising roles in mitigating obesity, the extent of our understanding of these drugs' implications for reproductive health remains inadequate.

This thorough review endeavors to aggregate and scrutinize the present corpus of evidence relating to the influence of these anti-obesity drugs on fertility. By exploring the prospective direct and indirect impacts of these pharmaceuticals on reproductive health, we aspire to illuminate their multifunctional roles extending beyond the sphere of weight regulation. In the female population, obesity has been correlated with menstrual abnormalities, infertility, and complications during gestation, frequently concurrent with an increased incidence of Polycystic Ovary Syndrome (PCOS). In the male counterpart, obesity has been associated with compromised semen quality and erectile function, often compounded by Metabolic Syndrome (MetS) and hormonal disequilibrium.

This exhaustive analysis seeks to equip physicians with a framework for refining their therapeutic approaches, appreciating the intricate interplay between obesity treatment and reproductive health. We aim to afford a broader and more nuanced comprehension of the complex interrelations between obesity, anti-obesity medications, and reproductive well-being. The intention is to empower healthcare practitioners with the insights necessary to deliver bespoke care strategies to those grappling with obesity and attendant fertility complications. Ultimately, this initiative augments our evolving understanding of the complex mechanisms operating at the juncture of obesity and fertility, thereby paving the way for future investigations in this critical field of study.

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MANUSCRIPT

1. Introduction

Overweight and obesity, characterized by excessive body fat accumulation, pose significant health risks. According to the World Obesity Federation in 2019, 13% of the global adult population were obese, 39% were overweight, and five million deaths were linked to obesity[1]. These conditions are the leading preventable causes of premature death worldwide and enhance the likelihood of developing chronic diseases such as hypertension, cardiovascular disease, dyslipidemia, and diabetes. In Mexico, a particularly concerning situation exists with 36.9% of adults being obese and 38.3% overweight. Furthermore, the prevalence of abdominal obesity in individuals aged 20 or older is 81.0%. Obesity is 45% more likely in women than men and 1.7 times higher in adults aged 40-59 than in the 20-39 age group. In adults with abdominal obesity, chronic diseases like diabetes, hypertension, dyslipidemia, and cardiovascular disease are more prevalent[2]. Among American countries, Mexico has the fifth-highest obesity prevalence. In recent years, overweight and obesity have become significant public health challenges in Mexico, affecting the population's well-being and the country's development. To address this epidemic, efforts need to intensify to ensure access to and availability of healthy food, promote healthier lifestyles, improve professional health training on overweight and obesity care, develop comprehensive interventions with a people-centered and planetary sustainability vision, enhance educational and awareness campaigns, foster environments promoting active lifestyles, and safeguard prevention and control efforts from conflicts of interest[3].

Obesity exerts a substantial impact on reproductive health in both males and females. In women, obesity has been associated with menstrual irregularities, infertility, and pregnancy complications. This excess adiposity has been linked to a higher prevalence of Polycystic Ovary Syndrome (PCOS), a commonly misunderstood endocrine disorder that results in ovulatory dysfunction, insulin resistance, and hyperandrogenism. In men, obesity can lead to diminished semen quality and erectile function. Beyond this, Metabolic Syndrome (MetS) marked by excessive adipose tissue is often associated with hormonal imbalances that can exacerbate infertility. Overarching these gender-specific effects, obesity can also disrupt hormonal production, thereby influencing overall reproductive function. It's worth noting that these conditions are not immutable; interventions such as weight loss, dietary changes, exercise, and pharmacological or surgical treatments have demonstrated the potential to restore hormonal balance, enhance semen quality, and improve sexual function. Nevertheless, the medical examination and history-taking of patients with obesity often overlook their reproductive health, indicating an urgent need for more comprehensive approaches in clinical practice. As a consequence of these reproductive challenges and their potential reversibility, obesity's impact on fertility underscores the need for more inclusive patient care and intensifying efforts in public health strategies aimed at combating obesity[4,5].

Several pharmaceutical agents have received approval for the management of obesity, and they have shown demonstrable efficacy in facilitating weight loss and ameliorating metabolic parameters. These pharmaceuticals encompass a wide array of mechanistic classes, including metformin, a biguanide that decreases hepatic glucose production; orlistat, a lipase inhibitor that reduces dietary fat absorption; and phentermine, a sympathomimetic amine that suppresses appetite. Moreover, the advent of glucagon-like peptide-1 (GLP-1) receptor agonists, such as liraglutide and semaglutide, have revolutionized obesity treatment due to their dual action in reducing appetite and inducing weight loss, in addition to their beneficial effects on glycemic control[6-10].

Nevertheless, despite their validated effectiveness in managing obesity, the investigation into these drug's direct and indirect impacts on reproductive function remains insufficiently thorough. Given the profound influence of obesity on reproductive health, the pressing need for rigorous exploration of these effects is starkly highlighted. It is essential not only to clarify these drug's roles in promoting weight loss but also to expand the research focus to encompass their potential effects on individual's reproductive capacities.

Considering this significant knowledge gap and the profound implications of obesity on reproductive health, the primary objective of this review article is to synthesize the available evidence regarding the effects of anti-obesity pharmacotherapy on fertility. By providing a comprehensive analysis of current data and identifying areas necessitating further research, we aim to foster a more integrative approach in the management of obesity, one that concurrently addresses weight reduction and fertility concerns. This approach will equip physicians with personalized, holistic care strategies, improving patient management and ultimately enhancing our understanding of the interconnected mechanisms of obesity and fertility.

2. Anti-obesity drugs and fertility

2.1 Metformin

Metformin is a pharmaceutical drug belonging to the biguanide class, and it is one of the most widely used oral antidiabetic agents globally. Metformin was first isolated in 1922, but it was not until 1957 that it was introduced in the United Kingdom for the treatment of type 2 diabetes mellitus (T2DM), and finally, in 1995, it was approved by the United States Food and Drug Administration (FDA) for the same purpose. This drug is derived from Galega officinalis, also known as "goat's rue" or "French lilac," a plant that has been used in traditional European medicine for centuries to treat various conditions, including the polyuria associated with diabetes[11].

Metformin, a widely prescribed treatment for type 2 diabetes mellitus, primarily operates by reducing hepatic glucose production and enhancing insulin sensitivity, leading to lower blood glucose levels. This is achieved not through the previously assumed inhibition of complex I activity, but rather via the inhibition of complex IV activity at clinically relevant concentrations. This inhibition prompts an increase in the cytosolic redox state, leading to a selective inhibition of glycerol-derived hepatic gluconeogenesis. Concurrently, at the cellular level, metformin activates AMP-kinase (AMPK), an enzyme pivotal to cellular energy homeostasis. The activation of AMPK results in a further decrease in hepatic gluconeogenesis and promotes glucose uptake by skeletal muscles. Consequently, these combined actions contribute to the glucose-lowering effects observed with metformin usage[12].

In addition to its approved use in the treatment of T2DM, metformin is also used off-label in various conditions such as polycystic ovary syndrome (PCOS), cancer, T1DM, anti-anging and some forms of insulin resistance. Regarding its use in managing obesity, metformin has shown to have a modest effect on weight loss in obese individuals, both diabetic and non-diabetic. Although weight loss is not its primary indication, it has been observed that metformin can promote weight loss by improving insulin sensitivity, which could have a feedback effect on reducing appetite and, therefore, caloric intake. Additionally, metformin may also have an effect on modulating the gut microbiota, which could play a role in regulating body weight. However, despite these positive effects, metformin is not officially approved for weight loss, and its use for this purpose should be carefully considered by a healthcare professional [13].

Metformin, a central agent in managing type 2 diabetes mellitus, is also known for its broad implications in reproductive health. It plays a significant role in both female and male reproductive physiology. In the context of Polycystic Ovary Syndrome (PCOS), a common disorder affecting 5-20% of women of reproductive age, metformin has been shown to improve ovarian cyclicity, mitigating the risk of gestational diabetes [14].

However, the effectiveness of metformin in improving hirsutism in adult PCOS women, particularly those with a Body Mass Index (BMI) of 25 kg/m^2 to 30 kg/m^2, may be less than oral contraceptive pills (OCPs). The certainty around this difference in effectiveness is less clear in BMI groups under 25 kg/m^2 and over 30 kg/m^2 [15].

Metformin's impact on hormonal profiles and ovarian function in PCOS is comparable to myo-inositol, as per findings by Azizi Kutenaei et al., they suggest that myo-inositol might improve fertility outcomes by moderating hyperandrogenism. Agrawal et al., 2019 concluded that the combination of metformin and myo-inositol is more effective in inducing ovulation in infertile PCOS women, resulting in significantly higher live birth rates[16,17].

In non-obese PCOS women, metformin usage is associated with a slight increase in clinical pregnancy rates compared to placebo, as discovered by Magzoub R et al., 2022. They also found metformin comparable to clomiphene citrate in terms of clinical pregnancy rates, though it has a higher risk of miscarriage. In addition, metformin may help preserve ovarian function and fertility during cyclophosphamide-based chemotherapy, which can cause severe gonadotoxicity, as suggested by Huang CC et al[18].

The study from Notaro, concluded that metformin is a suitable adjuvant medication for ovulation induction/stimulation in assisted reproduction therapies of both low and high complexity for PCOS women. The side effects are mainly mild, and there is no teratogenicity risk[19].

Metformin's effects also extend to male reproductive health, improving sperm concentration, motility, and morphology, particularly in obese individuals. Its treatment has shown to mitigate obesity-induced sperm abnormalities and improve serum testosterone and luteinizing hormone pulsatility in these individuals[20].

While metformin holds promising potential for both male and female fertility, it's essential to weigh potential risks and benefits individually. The safety of metformin during pregnancy remains controversial. Further research is needed to fully understand the complex interactions of metformin within the reproductive system and ascertain its long-term safety, especially during pregnancy.

2.2 Orlistat

Orlistat, is an anti-obesity drug that was first introduced into the market in 1998 by the pharmaceutical company Roche. It is a synthetic derivative of lipostatin, a molecule produced by the bacterium Streptomyces toxytricini, which exhibits an inhibitory effect on pancreatic and gastric lipases. Its mechanism of action focuses on the inhibition of these lipases, enzymes responsible for the breakdown of triglycerides in the intestine. By blocking this process, orlistat prevents the absorption of approximately one-third of the dietary fat, thereby favoring the reduction of total caloric intake[21].

The side effects of orlistat are directly related to its mechanism of action and include gastrointestinal discomfort, abdominal pain, flatulence, fatty diarrhea, and deficiencies in fat-soluble vitamins (A, D, E, and K) due to decreased fat absorption. The standard dose for adults is 120 mg three times a day, taken with meals[22].

Clinical studies have shown that orlistat can be effective in weight loss and in maintaining weight loss. A long-term 4-year study demonstrated that 37% of patients taking orlistat lost more than 5% of their initial body weight, and 19% lost more than 10%[23].

It is important to highlight that orlistat should be administered as part of a comprehensive weight loss program that includes diet, exercise, and behavior control, as it does not produce significant results on its own. Despite its widespread use for weight reduction, little is known about the effect of orlistat on reproductive health. In the following section, we will discuss the available studies on the relationship between orlistat use and fertility in both men and women.

Few studies have investigated the effects of Orlistat on fertility, primarily in overweight and obese women undergoing fertility treatments, such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), as well as in overweight and obese men.

One such study, conducted by Tong et al., involved 29 patients in the Orlistat group who completed 37 embryo transfer cycles. It was found that Orlistat intervention in overweight/obese infertile women undergoing IVF/ICSI increased the clinical pregnancy rate (59.46%) compared to the control group (39.47%). However, there was no significant difference in the live birth rate between the two groups[24].

A randomized controlled trial designed by Li et al., 2018, is currently underway to evaluate if preconception Orlistat treatment for 1-3 months can improve the live birth rate in overweight and obese women undergoing IVF and embryo transfer[25].

In a broader perspective, the FIT-PLESE trial, led by Legro et al., 2022, studied 379 women with obesity and unexplained infertility who underwent preconception lifestyle modifications. The results indicated that intensive lifestyle intervention and orlistat for weight loss did not improve fertility or birth outcomes compared to an exercise intervention without targeted weight loss[26].

As for male fertility, a study conducted by Suleiman JB et al., 2020, observed that Orlistat reversed infertility in obese male rats by targeting testicular lactate metabolism and sexual behavior, thereby suggesting that Orlistat may preserve the fertility potential in obese men[27]. Further investigation by the same group of researchers demonstrated that Orlistat attenuated the decline in steroidogenesis and spermatogenesis by up-regulating steroidogenic genes, indicating its potential to improve fertility in overweight/obese men[28].

Despite the positive results from these studies, it should be noted that the findings are not universally consistent, indicating that further research is needed to definitively determine the effects of Orlistat on fertility in both men and women.

2.3 Phentermine

Phentermine is a centrally acting stimulant and anorectic agent that is primarily used as a short-term adjunct in the management of obesity. It was first approved by the FDA in 1959 and has since been widely used in the United States and Mexico, retaining its place in the market due to its efficacy and safety profile, despite the emergence of various new weight loss drugs[29].

Phentermine works by stimulating the release of neurotransmitters in the brain, specifically norepinephrine, dopamine, and serotonin, leading to an increase in levels of these substances. This action results in appetite suppression and a feeling of fullness, thus decreasing food intake and promoting weight loss. It targets the hypothalamus, the part of the brain that controls hunger and satiety, effectively acting on the hunger axis[30].

While it has proven effective for weight loss when combined with a low-calorie diet and regular physical activity, phentermine can come with several mild and self-limiting side effects, which are well tolerated by patients, do not necessitate drug discontinuation. These may include increased heart rate, elevated blood pressure, dry mouth, insomnia, and constipation. Despite these side effects, the medication's benefits in the management of obesity often outweigh the potential risks for many patients[31].

Typically, phentermine is prescribed at a dosage of 15-30 mg per day, taken before breakfast or 1-2 hours after breakfast. It's important to note that it is recommended for short-term use, usually over a few weeks, as its efficacy tends to decrease with long-term use, and it can potentially lead to dependence[32].

When compared to other weight loss medications such as sibutramine, rimonabant, fenfluramine, and dexfenfluramine, phentermine has remained on the market, while these others have been withdrawn due to serious side effects. For instance, sibutramine was withdrawn due to an increased risk of cardiovascular events, while rimonabant was associated with psychiatric effects. Fenfluramine and dexfenfluramine were both associated with heart and lung problems[33-35].

Despite the wealth of data on phentermine's role as a weight control aid over several decades and its coverage in numerous studies, there remains a relative scarcity of information regarding its impact on fertility. This lesser-explored area merits further discussion with the objective of shedding light on how phentermine could potentially influence reproductive health, as specific information about its effects on both male and female fertility is still limited.

A retrospective study conducted by Chang, Lathi in an outpatient endocrinology clinic at a tertiary academic medical center examined the use of phentermine in women with obesity and infertility. It was observed that phentermine could induce clinically significant weight loss in obese women during the preconception period. The study found a pregnancy rate of 60% and a live birth rate of 49% following short-term phentermine use, indicating its positive effect on weight control, and therefore, potentially on fertility[36].

Furthermore, Jones et al. in a prospective controlled cohort study found no significant increase in rates of spontaneous pregnancy loss, or major or minor anomalies in the offspring of women who took phentermine/fenfluramine at the recommended daily dose during the first trimester of pregnancy[37].

On the other hand, Bajaj, Jain, and Stanford reported a case of a woman with childhood-onset obesity who temporarily gained 13-15 pounds during each controlled ovarian stimulation (COS) cycle for in vitro fertilization (IVF). This finding raises the need for further research on weight gain resulting from COS and how phentermine might play a role in mitigating this weight increase[38].

In a study conducted by Sarayani et al. prenatal exposure appeared to be significantly lower among phentermine-topiramate users under a Risk Evaluation and Mitigation Strategy (REMS) system, although pregnancy testing and contraceptive use seemed to be inadequate across all groups[39].

Elkind-Hirsch et al. carried out a study comparing phentermine/topiramate with other treatments, and found that dual therapy with EQW/DAPA resulted in the greatest weight and total body fat loss, along with significant improvements in mean blood glucose, insulin sensitivity, and insulin secretion measures, which could have implications for managing infertility in women with Polycystic Ovary Syndrome (PCOS)[40].

A study by Manakova, Kralova, and Hubičková Heringová in which pregnancies exposed to different appetite suppressants, such as sibutramine and phentermine, were prospectively followed, revealed no differences in pregnancy outcomes between the study and comparison groups[41].

In summary, there are no conclusive studies demonstrating that phentermine alone increases the likelihood of conception. However, given the well-documented relationship between weight and fertility, it is likely that women who achieve a healthier BMI with the aid of phentermine may have enhanced fertility. As for male fertility, most studies on phentermine have focused on its effect on weight control and its use in treating obesity, so further research in this area is needed.

2.4 GLP-1 receptor agonists (GLP-1RAs)

GLP-1 receptor agonists (GLP-1RAs), such as liraglutide and semaglutide, have been at the forefront of medical therapeutics since their inception. They are a class of medications that were initially developed to manage hyperglycemia in type 2 diabetes, leveraging the mechanism of glucagon-like peptide-1 (GLP-1), an endogenous hormone that enhances insulin secretion in a glucose-dependent manner[42].

The discovery and development of GLP-1RAs represent a significant milestone in the chronicles of diabetes therapy. Their mode of action involves mimicking the function of the natural incretin hormones, stimulating insulin secretion, and inhibiting glucagon production. This dual action results in improved glycemic control with a lower risk of hypoglycemia[43].

Additionally, GLP-1RAs have the unique property of delaying gastric emptying and promoting satiety, resulting in weight loss - an attribute that has expanded their therapeutic potential beyond diabetes to include obesity management. Notably, trials have consistently shown significant weight reduction, ranging from moderate to substantial, with doses of liraglutide (1.8-3.0mg/day) and semaglutide (0.5-1.0mg/week)[44].

GLP-1RAs, however, are not without side effects. Gastrointestinal disturbances, including nausea,

vomiting, and diarrhea, are the most common, but these usually subside with continued use. More serious concerns have been raised in recent years due to reports of suicide attempts, warranting careful patient monitoring and continued pharmacovigilance[45].

On the economic front, while the cost of these medications can be significant, it must be weighed against their broad-ranging therapeutic effects. Indeed, the marvel of these drugs extends beyond glycemic control and weight management to potential benefits on other organ systems, offering cardiovascular and renal protection[47].

However, as these drugs have only been in the market for a few years, long-term safety data and certain aspects of their effects, like their impact on fertility, are limited. The focus of the next section will be to explore the emerging data on the influence of GLP-1RAs on fertility, a topic that has been relatively unexplored due to their relatively recent introduction into the market.

The centrality of weight management in ameliorating fertility and pregnancy outcomes is a common theme across many studies. Cena, H., Chiovato, L., & Nappi, R.E illuminated a potential pathophysiological link between obesity, alterations in glucagon-like peptide-1 (GLP-1) kinetics, and the development of polycystic ovary syndrome (PCOS). Their research highlighted the promising outcomes of using GLP-1 receptor agonists (GLP-1 RAs), especially liraglutide, that included significant weight loss and testosterone reduction[48].

Insulin resistance is another crucial factor in the pathogenesis of PCOS as emphasized by Bednarz, K. et al. They posited that GLP-1 RAs could potentially counter insulin resistance and improve fertility through various mechanisms including modulation of glucose transporters in insulin-dependent tissues, reduction of inflammation and oxidative stress, and alteration of lipid metabolism[49].

Highlighting the broader metabolic impacts of GLP-1 RAs, Papaetis, G.S. & Kyriacou, A. discussed their beneficial effects in reducing weight, abdominal fat, and insulin resistance markers. They further underscored the ongoing research on the potential influence of these agonists on the hypothalamic-pituitary-gonadal axis[50].

In a 2019 study, Jensterle, M. et al. suggested that GLP-1 could be a pivotal modulator connecting the reproductive and metabolic systems. Their work provided preliminary evidence of improved menstrual regularity and increased fertility rates in overweight and/or obese women with PCOS treated with GLP-1 Ras[51].

Advancing this narrative, Violette, C.J. et al. explored the potential use of GLP-1 receptor agonists in fertility-sparing treatment for obese patients with malignant endometrial pathology. Their proposition entailed the potential enhancement of subsequent pregnancy chances through reductions in weight, inflammation, and insulin resistance by these agonists[52].

Corroborating the effectiveness of GLP-1 analogues, Reiser, E. et al. provided evidence that they, alongside metformin, were successful in regulating menstrual cycles in adolescents diagnosed with PCOS. Elkind-Hirsch, K.E. et al. further demonstrated that GLP-1 receptor agonists resulted in significant improvements in glucose regulation and insulin sensitivity, even in cases where equivalent BMI reductions were achieved with other treatments[53,54].

While GLP-1 receptor agonists show promise in improving fertility outcomes in the context of obesity, insulin resistance, and PCOS, further comprehensive studies are necessary to better assess their potential benefits and impacts.

Turning to male fertility, it appears that GLP-1 RAs also have a role to play. Rago et al. found that human sperm cells express GLP-1 receptors, impacting sperm function and metabolism. They suggested that these receptors could serve as new targets for GLP-1 incretin, thereby enhancing our understanding of male reproduction[55].

Gill and Mackey discussed the role of GLP-1 RAs, particularly liraglutide, as a treatment for obesity in women. This could have implications for male fertility, given that obesity is a known factor affecting reproductive health[56].

On a note of caution, Fontoura et al. reported an adverse effect of liraglutide on male reproductive function, raising concerns about its use in men, particularly those desiring fatherhood[57].

In a broader context, Ammar et al. suggested that GLP-1 RAs might offer new strategies for managing weight and infertility in obese men, a premise supported by Zhang et al.'s study that demonstrated the attenuating effects of a GLP-1 RA, exenatide, on reduced sperm quality and increased testicular inflammation in obese mice[58,59].

Overall, GLP-1 RAs could significantly impact both male and female reproductive health. However, a more in-depth understanding of these effects is needed to develop optimal treatment strategies.

2.5 Dual agonists of GLP-1 and GIP

Dual agonists of GLP-1 and GIP, like tirzepatide, represent the next wave of innovation in the realm of

diabetes management and weight loss therapy. Exploiting the synergistic actions of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), these novel therapeutics promise to reshape the landscape of metabolic disease treatment[60].

Both GLP-1 and GIP are incretin hormones secreted by the gut in response to nutrient intake. While GLP-1's role in enhancing glucose-dependent insulin secretion and inducing satiety is well-established, GIP was initially known for its insulinotropic effect. However, recent evidence points to its role in lipid metabolism and energy homeostasis, which has been leveraged in the development of dual GLP-1/GIP agonists[61].

The action of these dual agonists, therefore, combines the benefits of both incretin pathways: improved glucose control, weight loss, and potential benefits in lipid metabolism. Notably, early-phase trials with tirzepatide have shown substantial weight reduction and significant improvement in glycemic control[62].

As a relatively new development, data on the side effects of dual GLP-1/GIP agonists are limited but are anticipated to align with those of GLP-1 agonists, including gastrointestinal disturbances such as nausea, vomiting, and diarrhea. However, close patient monitoring remains a priority due to the novel nature of this medication class[63].

As tirzepatide is yet to hit the market, the cost implications are uncertain. However, the potential broad-spectrum benefits extending beyond glycemic control and weight loss, to potentially improving other metabolic parameters, are promising. Comparatively, dual GLP-1/GIP agonists like tirzepatide appear to offer more robust weight loss compared to GLP-1 monoagonists, suggesting a potential edge in obesity management[64].

However, as with any novel therapy, there is a significant knowledge gap regarding the long-term safety and certain aspects of its effects, such as fertility. In the following section, we will explore what is known about the impact of dual GLP-1/GIP agonists on fertility, a topic that has been relatively untouched given the novelty of these agents.

Research data so far has shown both promise and potential concerns for the use of tirzepatide in relation to fertility and reproductive health.

The official prescribing information for tirzepatide, marketed as Mounjaro, provides key information on the potential effects on fertility. According to the FDA-approved label, in studies conducted in rats, tirzepatide did not show any effects on sperm morphology, mating, fertility, or conception in males. In female rats, however, there were changes in reproductive parameters including prolonged diestrus and decreased numbers of corpora lutea, implantation sites, and viable embryos. These changes were considered secondary to the pharmacological effects of tirzepatide on food consumption and body weight. The same prescribing information also highlighted a significant interaction between tirzepatide and oral hormonal contraceptives. In the presence of a single dose of tirzepatide 5 mg, the maximum concentration (Cmax) of ethinyl estradiol, norgestimate, and norelgestromin was reduced by 59%, 66%, and 55%, respectively. The total amount of drug absorbed, represented by the area under the curve (AUC), was reduced by 20%, 21%, and 23%, respectively. Therefore, patients using oral hormonal contraceptives are advised to switch to non-oral contraceptive methods for 4 weeks after initiation and 4 weeks after each dose escalation with tirzepatide[65].

Anala et al. proposed tirzepatide as a potential treatment for PCOS. Although weight loss facilitated by glucagon-like peptide-1 receptor (GLP-1R) agonists has been found to improve the metabolic features of PCOS, side effects including gastrointestinal discomfort can hinder patient compliance. Tirzepatide may benefit obese patients with PCOS who have metabolic syndrome. However, its effectiveness in PCOS patients of normal weight is uncertain[66].

Tirzepatide shows potential as a treatment in specific aspects of reproductive health, particularly in relation to metabolic issues associated with PCOS. However, the drug's interactions with oral hormonal contraceptives and potential effects on fertility underscore the need for careful consideration and additional research.

Conclusion

The intersection of obesity, metabolic disease, and reproductive health continues to be a significant focus of research. In this review, we have explored the current evidence for the impact of five key classes of anti-obesity medications on reproductive parameters: metformin, orlistat, phentermine, GLP-1 receptor agonists, and dual GLP-1 and GIP agonists.

Metformin, a pharmaceutical stalwart in managing type 2 diabetes mellitus, has displayed extensive implications for reproductive health. Notably, it has been beneficial in managing Polycystic Ovary Syndrome (PCOS), a common disorder affecting a significant percentage of women of reproductive age. However, its efficacy compared to other treatments such as oral contraceptive pills (OCPs) may vary based on the patient's body mass index. Interestingly, studies suggest the combination of metformin and myo-inositol could improve fertility outcomes in infertile women with

PCOS. In men, metformin has shown promising improvements in sperm quality, especially in obese individuals. Yet, it is vital to individually weigh the potential risks and benefits, particularly given the ongoing controversy regarding metformin's safety during pregnancy.

Orlistat, an inhibitor of pancreatic and gastric lipases, reduces total caloric intake by preventing the absorption of dietary fat. The drug can effectively promote weight loss and maintain it, but it may also lead to deficiencies in fat-soluble vitamins. Research into its effects on fertility is sparse, but emerging evidence suggests potential benefits for obese women undergoing fertility treatments and men. However, the consistency of findings across studies is yet to be established, calling for further research to definitively elucidate Orlistat's impact on fertility.

Phentermine, a stimulant used for short-term obesity treatment, promotes weight loss by suppressing appetite through neurotransmitter release. Although well-documented for its weight control effectiveness, little is known about its direct influence on fertility. The drug, often combined with a low-calorie diet and exercise, is usually prescribed for a short term due to its potential for dependency. Side effects are typically mild and temporary. Unlike some weight loss drugs, phentermine remains available due to its effectiveness and safety. In comparison to other weight loss medications, phentermine has remained on the market due to its efficacy and safety profile, while others have been withdrawn due to serious side effects. However, despite the extensive data supporting phentermine's role in weight control, specific research on its direct impact on both male and female fertility is limited. The indirect influence on reproductive health through significant weight loss and improvement in metabolic parameters is plausible, though the exact impact remains under-explored and warrants further investigation.

GLP-1 receptor agonists (GLP-1RAs) like liraglutide and semaglutide demonstrate significant weight reduction and improved metabolic aspects of Polycystic Ovary Syndrome (PCOS), indicating potential reproductive benefits. Originating as type 2 diabetes treatments, their dual action improves glycemic control and aids in weight loss, broadening their utility to obesity management, despite potential side effects including gastrointestinal disturbances and serious mental health concerns. While effective for hyperglycemia management and weight loss, data on their fertility impacts are sparse. Nevertheless, they've shown promise in addressing weight management and insulin resistance, key to PCOS, and some evidence suggests improved menstrual regularity and fertility rates in overweight or obese women with PCOS. They have also been proposed for fertility-sparing treatments in obese patients with endometrial pathology. Regarding male fertility, early studies suggest a potential role for GLP-1RAs, although concerns exist about potential negative impacts on reproductive function. In summary, GLP-1RAs, potentially influencing both male and female reproductive health by addressing metabolic factors, obesity, and insulin resistance, warrant further investigation to ascertain their fertility effects.

Finally, Dual GLP-1 and GIP agonists, such as tirzepatide, are emerging innovations in the treatment of diabetes and weight loss, offering significant improvements in glucose control and weight reduction. However, there are unknowns regarding side effects, long-term safety, and impacts on fertility. Tirzepatide shows potential for treating Polycystic Ovary Syndrome (PCOS) by facilitating weight loss, a key factor in managing PCOS. But its interactions with oral contraceptives and unclear effects on fertility demand more research. While tirzepatide appears to provide stronger weight loss outcomes than GLP-1 monoagonists, comprehensive studies are necessary to fully understand its safety, efficacy, and broader implications on reproductive health.

Pharmaceutical interventions like Orlistat, Metformin, Phentermine, GLP-1 agonists, and dual GLP-1/GIP agonists show promising potential in managing metabolic disorders and obesity, with possible positive impacts on reproductive health. However, current knowledge about their direct effects on fertility is limited and often ambiguous. It's vital that future research is directed towards closing this information gap, to better understand their full impact and safely broaden their therapeutic applications in reproductive health.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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Relationship of morphology and chromatin integrity of sperm in aneuploid blastocyst development: embryos fertilized with sperm diagnosed with teratozoospermia.

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ABSTRACT

Objective:

Evaluate whether the presence of aneuploid blastocysts is associated with sperm morphology and fragmentation.

Methods:

Retrospective, observational and cross-sectional study, which included 352 embryos in blastocyst stage, obtained by intracytoplasmic sperm injection (ICSI) from 131 cycles of patients with implantation failure and who decided to perform preimplantation genetic study of aneuploidy (PGT-A) to the embryos that were transferred to the uterus, in order to improve the implantation rate.

Results:

Of the embryos obtained from donated oocytes, only those fertilized with semen diagnosed with teratozoospermia presented aneuploidy (26.6%). The rate of aneuploid embryos was similar when own oocytes were fertilized with semen diagnosed with normozoospermia or teratozoospermia (38.4 vs 37.07%). Finally, no relationship was observed between chromatin damage and sperm morphology.

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Conclusions:

In patients who fertilize their oocytes with spermatozoa from samples diagnosed with normozoospermia or teratozoospermia, the rate of aneuploid blastocysts will depend mainly on the female factor, this does not rule out the possibility of aneuploid embryos due to the male factor. Different from what was seen in donated oocytes, where embryos obtained from semen diagnosed with teratozoospermia presented a higher rate of aneuploidy.

KEYWORDS: Recurrent implantation failure; recurrent pregnancy loss, sperm morphology, sperm chromatin, PGT-A.

MANUSCRIPT

Introduction

In recent years there has been an increase in female infertility worldwide; in Mexico, rates of up to 17.5% have been reported, which coincides with various international studies [1, 2]. In addition, it is estimated that 1.5 million couples in the world have been infertile for more than 12 months without using any contraceptive method. This represents 15-20% of couples who have approached an assisted reproduction clinic [3] due to difficulty in conceiving.

In addition, chromosomal abnormalities originating in cell duplication by meiosis or mitosis are one of the main causes of infertility, as they have been associated with embryo implantation failure as well as gestational loss. Due to the increase in maternal age. That is to say, advanced maternal age (AMA) the risk of having aneuploid embryos, high risk of miscarriages, implantation failure in in vitro fertilization (IVF) cycles as well as in intracytoplasmic sperm injection, fetal malformations and even the birth of babies with chromosomal disorders increases.

In view of this situation, assisted reproduction centers have developed technology to evaluate embryos before their transfer to the uterus, which makes it possible to rule out those that present genetic or chromosomal alterations, such as the presence of aneuploidies (monosomies and trisomies in any of the 23 pairs of chromosomes), an example of which is the preimplantation genetic test (PGT) [4].

PGT is a test developed to analyze DNA from the polar bodies of oocytes or embryos at the third day (cleavage stage) or fifth day of development (blastocyst). PGT was introduced more than 30 years ago as a form of prenatal genetic diagnosis to prevent the transmission of genetic disorders in patients with monogenic diseases (PGT-M), the transmission of chromosomal rearrangements (duplications, deletions, inversions and translocations) (PGT-SR) and to prevent the transfer of aneuploid embryos with monosomies or trisomies (PGT-A), which are one of the causes of implantation failure and gestational loss [5].

In order to identify aneuploid embryos, blastocysts are currently biopsied, taking five to ten cells from the trophectoderm, with the advantage of giving more accurate results in PGT, because a larger sample is obtained since they are larger embryos, which generates less impact on viability, compared to biopsies in embryos from the third day (cleavage stage) [5, 6].

Since the implementation of PGT, a large number of technologies have been used for genetic evaluation, such as fluorescence in situ hybridization (FISH), comprehensive chromosome screening (CCS) technology, including real-time quantitative PCR (qPCR), array comparative genomic hybridization (aCGH) and next-generation sequencing (NGS). NGS is the technology currently employed and is of great importance for its high sensitivity in the identification of aneuploidies, while it is characterized by: high throughput, low cost, high sensitivity and specificity; in addition, it can also be used for the identification of translocations and certain point mutations [5, 7].

The objective of this study is to evaluate whether the presence of aneuploid blastocysts is associated with sperm morphology and sperm chromatin integrity.

Material and Method

Retrospective, observational and cross-sectional study, which included 352 embryos in blastocyst stage, obtained by intracytoplasmic sperm injection (ICSI) from 131 cycles of patients with implantation failure who attended the PRONATAL clinic located inside the Hospital Bité Médica in Mexico City, in the year 2021; and who in the present study decided to perform preimplantation genetic study of aneuploidy (PGT-A) to the embryos that were transferred to the uterus, in order to improve the implantation rate.

For this study, four groups were formed:

OWN-N: Blastocysts obtained from oocytes aspirated from the same patient who will undergo embryo transfer and fertilized by ICSI with sperm diagnosed with normozoospermia.

PROPIOS-T: Blastocysts obtained from oocytes aspirated from the same patient who will undergo embryo transfer and fertilized by ICSI with sperm diagnosed with teratozoospermia.

OVODON-N: Blastocysts obtained from donated oocytes and fertilized with sperm diagnosed with

normozoospermia from the couple that underwent embryo transfer.

OVODON-T: Blastocysts obtained from donated oocytes and fertilized with semen diagnosed with teratozoospermia of the couple that performed the embryo transfer.

The anthropometric data and the procedures performed in the assisted reproduction laboratory were obtained from the patient's history, which was filled out by the nursing staff, as well as embryologists and physicians during the first consultation.

Evaluation of sperm quality (spermatobioscopy)

All sperm samples evaluated were obtained by masturbation in a dedicated area conditioned for masturbation, with an abstinence period of three to seven days. Macroscopic parameters such as volume, appearance (color), liquefaction, viscosity and pH were evaluated, as well as other microscopic parameters such as: concentration, motility (progressive, in situ and immotile), morphology and fragmentation index.

Both sperm morphology and chromatin integrity were assessed using Diff-Quik (Dade Behring Inc., Newark, NJ, USA), composed of methanol (fixative), eosin (dye that stains basic proteins red) and a thiazine-type stain (that stains DNA blue).

Ten µl of the semen sample was placed on a slide and allowed to air dry. The slides were then dipped sequentially for 10 to 20 seconds in each kit solution and then quickly dipped in water to remove excess dye. The slides were allowed to air dry and observed under a brightfield microscope. Chromatin integrity was analyzed using the following staining categories: spermatozoa with light heads/nuclei (normal staining) and spermatozoa with dark heads/nuclei (abnormal staining). To determine the percentage of dark sperm nuclei, 200 cells per sample were counted in four different fields [8]. Sperm morphology was evaluated using 2015 WHO criteria.

Controlled ovarian stimulation (COS) and embryo morphology

Controlled ovarian hyperstimulation was performed with the use of a gonadotropin-releasing hormone (GnRH) antagonist on the basis of a short protocol.

The protocol was with administration on day 1 or 2 of gonadotropins (Merional, Merapur or Pergoveys) for 9 days, beginning the application of the antagonist on day 6 or 7 (Cetrotide), until the presence of at least 3 follicles larger than 17mm was observed. Moment in which hCG was applied (day 11 or 12) (Ovidrel). Oocyte retrieval was performed 34 to 36 hours later (follicular aspiration).

Intracytoplasmic sperm injection was used in all procedures. Thus, all embryos were "cultured" until the blastocyst stage was reached. According to Gardner's criteria[9], the morphological scoring of the blastocyst was based on three components: blastocyst expansion, inner cell mass and trophectoderm development.

Preimplantation genetic study of aneuploidy (PGT-A)

A trophectoderm biopsy was performed on blastocysts on the fifth day and sent to a specialized laboratory for PGT-A, which is carried out using the massive sequencing technology (NGS). In addition, for library preparation, the Ion ReproSeqPTM PGS kit is used in order to perform 24-chromosome aneuploidy analysis together with the Ion ChefPTM System (Thermo Fisher Scientific, USA). 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). The biopsied blastocysts were vitrified, awaiting PGT-A results for euploid embryo transfer.

Inclusion Criteria

Couples with:

- Reproductive age.
- Implantation failure ≥ 1.
- Complete medical history.
- Embryos with PGT-A.
- Complete spermogram.
- Assessment of sperm chromatin integrity

Exclusion criteria

- Infertility associated with thrombophilias, anatomical factor and autoimmune factor.
- Study of altered endometrial receptivity.
- Couples who did not accept their inclusion in this study.

Statistical analysis

Parametric data such as anthropometric history and spermogram results are reported as mean ± standard deviation (SD) and the statistical difference was evaluated with Student's t-test. Non-parametric data, such as complications that occurred during pregnancy, aneuploid blastocysts and aneuploidies were reported as percentages and the statistical difference was analyzed with Chi2 test. In both cases the SPSS statistical package was used in its version 25, it was significant p≤0.05.

Results

This study included 352 embryos obtained from 131 cycles that were separated into 4 groups. It was observed that in the anthropometric data DONATION-T presented a statistical increase in age (45.3±2 vs 37.6±4, p≤0.05), weight (70.6±19.8 vs 62.02±5.3, ≤0.05) and height (1.70±0.1 vs 1.56±0.03, p≤0.05) when compared to DONATION-N. As for BMI it was statistically higher in DONATION-N compared to DONATION-T (25.3±3.02 VS 23.9±5.2, p≤0.05). in the case of OWN-T this showed lower in weight (60.1±8.9 vs 63.3±9.8) and BMI (22.9±2.6 vs 24.02±3.2, p≤0.05) compared to OWN-N (**Table 1**).

	OWN-N	OWN-T	DONATION-N	DONATION-T
Patients (N)	33	69	8	21
Blastocysts with PGT-A (N)	84	198	20	52
Age (median±SD)	37.6±4	36.7±3.3	37.2±3.7	45.3±2**
Weight (median±SD)	63.3±9.8	60.1±8.9*	62.02±5.3	70.6±19.8**
Height (median±SD)	1.62±0.05	1.61±0.05	1.56±0.03	1.70±0.1**
BMI (median±SD)	24.02±3.2	22.9±2.6*	25.3±3.02	23.9±5.2**

Table 1. **Anthropometric data of the mother**
**Statistical difference of OWN-N with OWN-N and ** Statistical difference of DONATION-N with DONATION-T Student's t-test (p≤0.05).*

In the case of the patients included in the study, OWN-T showed a statistical decrease in RIF (10.1 vs 19.04 %, p≤0.05) and a statistical increase in IF (89.8 vs 80.9%, p≤0.05) when compared to OWN-N. In parallel, DONATION-T showed a numerical increase in RIF (11.5 vs 0%) and a numerical decrease in IF (100 vs 88.4%), compared to DONATION-N (**Table 2**).

In the IVF cycle, OWN-T and DONATION-T reported higher statistical prevalence of blastocysts, compared to their counterparts OWN-N (4.2±2.1 vs 3.6±1.2, p≤0.05) and DONATION-N (4.3±1.7 vs 2.3±0.8, p≤0.05). Of these embryos, the group with the highest FET rate was DONATION-T (50%), compared to OWN-N (30.9%), OWN-T (30.3%) and DONATION-N (30.7%). Implantation rate was numerically higher in OWN-N and DONATION-N, compared to OWN-T (61.5 vs. 46.6%) and DONATION-T (80 vs. 80%). Similarly OWN-N and DONATION-N, showed numerically increased clinical pregnancy rate, when compared to OWN-T (61.5 vs 46.6%) and DONATION-T (80 vs 80%) (**Table 2**).

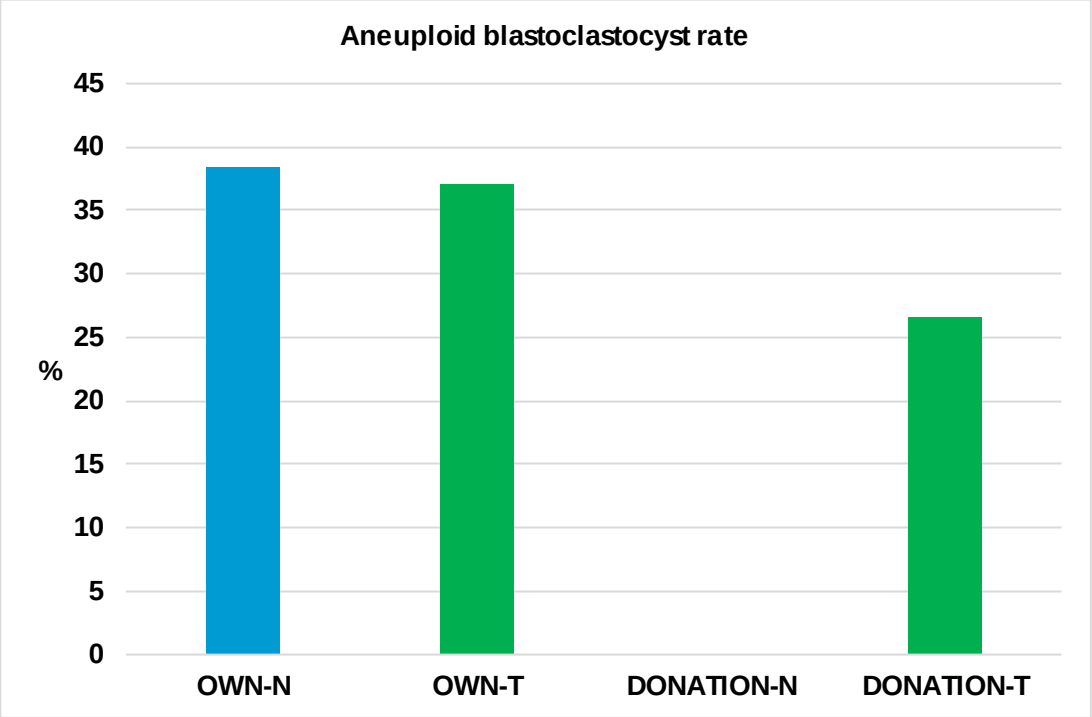
	OWN-N	OWN-T	DONATION-N	DONATION-T
Blastocysts with PGT-A (N)	84	198	20	52
	Female factor			
AMA, % (n/N)	66.6 (56/84)	63.6 (126/198)	60 (12/20)	53.8 (28/52)
RIF, % (n/N)	19.04 (16/84)	10.1 (20/198) *	0	11.5 (6/52)
IF, % (n/N)	80.9 (68/84)	89.8 (178/198) *	100 (20/20)	88.4 (46/52)
	Male factor			
Volume	2.5±1.8	2.2±1.7	2.1±1	1.5±0.5**
Concentration	79.3±44.6	61.1±28*	128.8±38.9	51.5±12.2**
Sperm (PM)	50.2±16.5	43.7±19.7*	63.1±5.8	44.3±11.1**
Sperm (NPM)	14.7±4.7	16.4±8.8*	14.6±2.1	17.4±7.9**
Sperm immobile	35±12.4	38.6±18.7*	22.2±7.5	38.2±10.4**
Morphology	4	2.1±0.5*	4	2.7±0.9**
Integridad de la cromatina	12.5±3.4	13.2±2.6*	13.1±3.5	12.8±1**
	Ciclo de FIV			
MII	12.2±5.7	13.8±4.9	10.7	11.4±4.9
Fecundation	9.8±3.7	10.9±4.2	8.6±2.9	9.3±4.3
Blastocyst	3.6±1.2	4.2±2.1*	2.3±0.8	4.3±1.7**
FET	30.9 (26/84)	30.3 (60/198)	50 (10/20)	30.7 (16/52)
Embryo by transfer	1.05±0.2	1.17±0.4	1	1
Implantation rate	69.2 (18/26)	46.6 (28/60)	80 (8/10)	50 (8/16)
Clinical pregnancy	61.5 (16/26)	46.6 (28/60)	80 (8/10)	50 (8/16)

Table 2. Euploid blastocyst (PGT-A), female and male factor; and success rate.

AMA: Advanced Maternal Age, IF: Implantation failure, RIF: Recurrent Implantation Failure, Sperm (PM): Sperm with Progressive Motility and Sperm (NPM): Sperm with Non-Progressive Motility, MII: Metaphase II oocyte, FET: Frozen Embryo Transfer. *Statistical difference of OWN-T with OWN-N, **Statistical difference of DONATION-T with DONATION-N. *Student's t-test (parametric date) and Chi^2 test (non parametric date) (p≤0.05).*

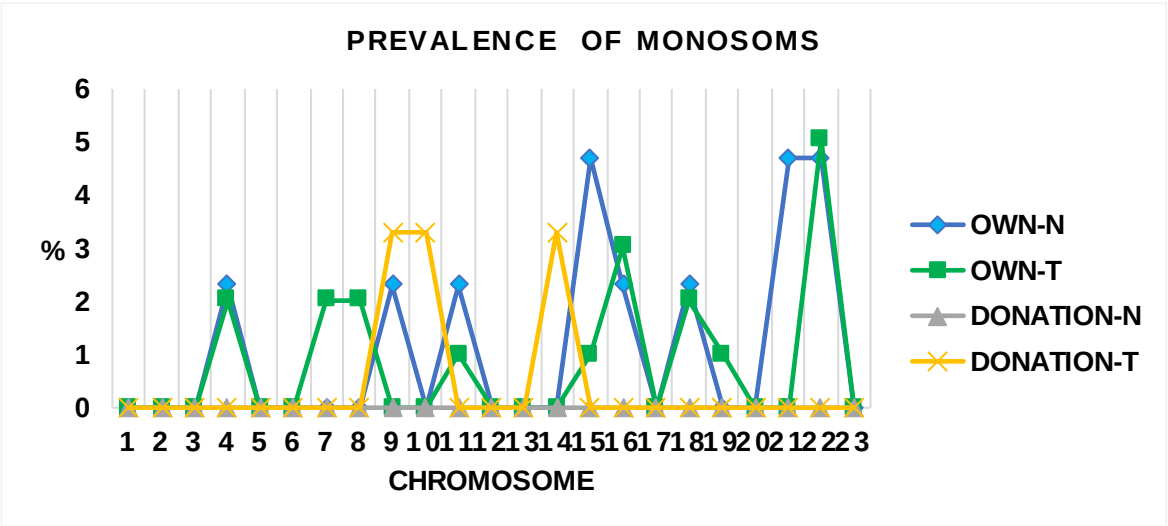
As for spermogram results: OWN-T showed statistical decrease of Volume (61.1±28 VS 79.3±44.6, p≤0.05), sperm (PM) (43.7±19.7 vs 50. 2±16.5, p≤0.05) and morphology (2.1±0.5 vs 4, p≤0.05); and statistically increased DNA fragmentation (13.2±2.6 vs 12.5±3.4, p≤0.05) compared to OWN-N. In turn, DONATION-T presented a statistical decrease in volume (1.5±0.5 vs 2.1±1, p≤0.05), concentration (51.5±12.2 vs 128.8±38.9, p≤0.05), sperm (PM) (44. 3±11.1 vs 63.1±5.8, p≤0.05), morphology (2.7±0.9 vs 4, p≤0.05) and DNA fragmentation (12.8±1 vs 13.1±3.5, p≤0.05), compared to Donation-N (**Table 2**).

In parallel, no significant difference was found in the rate of aneuploid embryos between OWN-N and ONW-T (38.4 and 37.07%), different from what was observed in DONATION-T, which presented a higher rate of aneuploidy compared to DONATION-N (0 vs 26.6%) (**Graph 1**).



Graph 1. Shows the prevalence of aneuploidies in the different groups of patients.

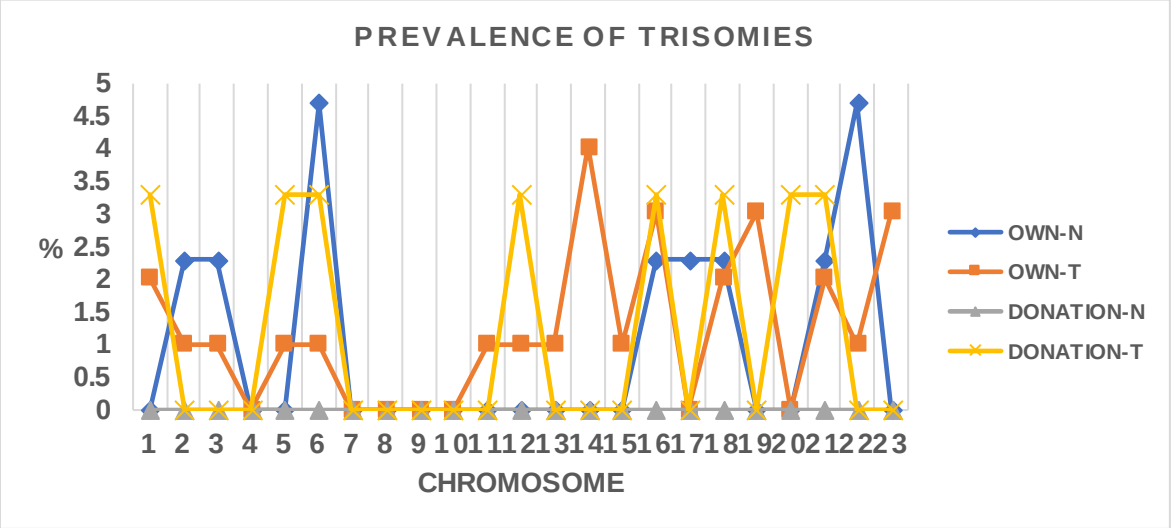
In addition, when the type of aneuploidy was analyzed, it was found that DONATION-N did not present monosomies and DONATION-T only presented monosomies in chromosome 9 (3.3%), 10 (3.3%) and 14 (3.3%). The case of OWN-N was different since it showed a higher number of chromosomes affected by monosomies [2 (2.3%), 9 (2.3%), 11 (2.3%, 15 (4.7%), 16 (2.3%), 18 (2.3%), 15 (4.7%), 16 (2.3%), 18 (2.3%) and 14 (3.3%). 3%), 18 (2.3%), 21 (4.7%) and 22 (4.7%)], similar to OWN-T [4 (2.02%), 7 (2.02%), 8 (2.02%), 11 (1.01%), 15 (1.01%), 16 (3.03%), 18 (2.02%), 19 (1.01%) and 22 (5.05%) (**Graph 2**)



Graph 2. Shows the prevalence of monosomies in each of the 23 chromosomes. Evaluation performed in OWN-N, OWN-T, DONATION-N and DONATION-T.

It was also found that DONATION-N did not present trisomies in any chromosome, compared to DONATION-T who had trisomy in chromosome 1 (3.3%), 5 (3.3%), 6 (3.3%), 12 (3.3%), 16 (3.3%), 20 (3.3%) and 21 (3.3%). Finally, both ONW-N [chromosome 4 (2.3%), 9 (2.3%), 11 (2.3%), 15 (4.7%), 16 (2.3%), 18 (2.3%), 21 (4.7%) and 22 (4.7%)] and OWN-T [4 (2. 02%), 7 (2.02%), 8 (2.02%), 11 (1.01%), 15 (1.01%), 16 (3.03%), 18 (2.02%), 19 (1.01%) and 22 (5.05%)], had trisomies on various chromosomes (**Graph 3**).

compared to OWN-N. As well as the decrease in volume, concentration and sperm (PM) in the case of DONATION-T when compared to DONATION-N. In addition, the samples are still considered normal only in these parameters, according to the criteria of the World Health Organization (WHO) according to the latest revision of the laboratory manual for the review and processing of human semen, published in 2021 [13]. Likewise, to date there are no studies showing that the numbers obtained in these parameters are associated with increased aneuploidy [14, 15, 16].



Graph 3. Shows the prevalence of trisomies in each of the 23 chromosomes. Evaluation performed in OWN-N, OWN-T, DONATION-N and DONATION-T.

Discussion

Advanced maternal age plays a crucial role in embryo quality and has long been known to be related to the increase of aneuploid embryos in IVF cycles [10]. In this study, it was found that the mean of the four groups exceeded 35 years of age and DONATION-T, presented the highest age (45.3±2 vs 37.6±4, 36.7±3.3 and 37.2±3.7, p≤0.05), weight (70.6±19.8 vs 63. 3±9.8, 60.1±8.9 and 62.02±5.3, p≤0.05) and height (1.70±0.1), a relationship that did not generate overweight unlike DONATION-T, who resulted with higher BMI (25.3±3.02 vs 24.02±3.2, 22.9±2.6 and 23.9±5.2). This may be one of the reasons why these patients have infertility problems, since overweight and obesity are related to the decline in reproductive outcomes in IVF cycles with a decrease in clinical pregnancies and live births and, on the contrary, an increase in spontaneous abortions, without being related to the increase in aneuploid embryos [11, 12].

Regarding the male factor, we observed a decrease in seminal parameters such as sperm concentration (PM) in the case of OWN-T when

On the other hand, this study shows that OWN-N and OWN-T have similar prevalence of aneuploidies (38.4 vs 37.07%) (Graph 1), as well as they coincide in the presence of monosomies in chromosome 4 (2.02 vs 2.02%), 11 (2.02 vs 1.01%), 15 (1.01 vs 4. 7%), 16 (2.3 vs 3.03%), 18 (2.02 vs 2.02%) and 22 (4.7 vs 5.05%) and with monosomies other than OWN-N on chromosome 9 (2.3%), 11 (2.3%) and 21 (4.7%) compared to OWN-T which showed monosomies on chromosome 6 (2.2%), 8 (2.2%) and 19 (1.01%) (**Graph 2**).

In parallel, OWN-N and OWN-T coincide with the presence of trisomy 2 (2.3 vs 1.01%), 3 (2.3 vs 1.01%), 6 (4.7 vs 1.01), 16 (2.3 vs 3.3%), 18 (2.3 vs 3.3%), 21 (2.3 vs 2.03), 22 (4.7 vs 1. 01); and differ in the presence in OWN-N of trisomy 17 (2.3%) with OWN-T presenting trisomy 1 (2.02%), 5 (1.01%), 11 (1.01%), 13 (1.01%), 14 (4.04%), 15 (1.01%),19 (3.03%), 22 (1.01%) and 23 (3.03%) (Graph 3). This is different from what was reported by Kiseleva Y. et al., 2017, in study that included 46 couples, in which the male presented sperm morphology ≤4% and when compared with control group with morphology >4, it

was found that embryos obtained from own eggs and semen diagnosed with teratozoospermia presented higher prevalence of autosomal monosomies (46.2 vs 26.4%), autosomal trisomies (48.5 vs 25.5%) and sex chromosome trisomies (7.2 vs 3.4%) [17]. Similarly, Rodriguez J. et al., 2016, it was observed that the rate of aneuploid embryos obtained from own eggs and semen with morphology less than 2% was higher, when evaluating almost 8000 embryos [18].

In the case of DONATION-N, there were no aneuploidies, in contrast to DONATION-T, which presented a rate of 26.6%. In the latter, monosomy was observed in chromosome 9 (3.3%), 10 (3.3%) and 14 (3.3%) (Graph 2); and trisomy in chromosome 1 (3.3%), 5 (3.3%), 6 (3.3%), 12 (3.3%), 16 (3.3%), 18 (3.3%), 20 (3.3%) and 21 (3.3%) (Graph 3). This could indicate that fertilization of donated eggs with semen diagnosed with teratozoospermia could be associated with increased aneuploid blastocysts, coinciding with Coban O. et al., 2018, who in study that included 1165 ovodonation embryos that were fertilized with semen diagnosed with teratozoospermia (1 to 4, by strict kruger criteria), observed the decrease in morphology presents inversely proportional relationship with the increase of aneuploidies (monosomies and trisomies of chromosome 13, 18, 21 and 23) [19].

As can be seen, aneuploidies are not only associated with the female factor, but there is also a certain percentage related to the male factor. It is known, as a result of abnormal chromosome segregation, which occurs during meiosis and can lead to aneuploidy in both female and male gametes by removing and including an extra copy of some chromosome, and the most frequent is aneuploidy in spermatozoa of approximately 4.5% and oocytes in approximately 20% [19].

There was an increase in fragmentation index with respect to chromatin integrity or sperm DNA fragmentation (SDF) index in OWN-T and DONATION-N compared to their counterpart OWN-N (13.2±2.6 vs 12.5±3.4, P≤0.05) and DONATION-T (13.1±3.5 vs 12.8±1, p≤0.05). Thus, all four groups showed sperm fragmentation index <15% which is classified with good fertilization potential [20, 21], preventing to observe relationship of SDF and development of aneuploid embryos. For its part, SDF can be caused by extrinsic factors such as: exposure to heat, smoking, environmental pollution and chemotherapy; as well as intrinsic factors, such as defects in germ cell maturation, abortive apoptosis and oxidative stress, all resulting in male infertility, as well as decreased fertilization rate and embryo development [22, 23, 24]. Furthermore, a large number of studies agree that there is no association of SDF with the development of aneuploid embryos [23, 24].

On the other hand, in OWN-T (4.2±2.1) and DONATION-T (4.3±1.7) a higher blastocyst rate was obtained in comparison with OWN-N (3.6±1.2) and DONATION-T (2.3±0.8), coinciding with previous studies, where the selection of spermatozoa with normal morphology by microscopy during ICSI, allows excellent results even with samples with severe teratozoospermia [26, 27]. In addition, we confirmed that the embryonic development of OWN-T and DONATION-T was not affected by the fragmentation index because it was low in all four groups.

Finally, the implantation and clinical pregnancy rate was lower in OWN-T (46.6 and 46.6%) and DONATION-T (50 and 50%) compared to OWN-N (69.2 and 61.5%) and DONATION-N (80 and 80%). We still do not know the exact mechanisms by which embryos from semen diagnosed with teratozoospermia presented a lower implantation rate. But there are studies which suggest that spermatozoa selected for ICSI may have undetectable structural alterations at 400X such as nuclear vacuoles, which decreases the pregnancy and implantation rate. Therefore, more sensitive techniques such as intracytoplasmic injection of morphologically selected spermatozoa (IMSI) are recommended, where better implantation rates have been obtained when compared with ICSI [28].

CONCLUSIONS

When intracytoplasmic sperm injection (ICSI) is performed by selecting normal spermatozoa from samples diagnosed with teratozoospermia, the rate of aneuploid embryos is increased when they are used to fertilize donor oocytes.

In addition, the rate of aneuploid embryos is similar when fertilizing own oocytes with semen diagnosed with normozoospermia or teratozoospermia.

One the other hand, in patients who fertilize their oocytes with spermatozoa from samples diagnosed with normozoospermia or teratozoospermia, the rate of aneuploid blastocysts will depend mainly on the female factor. This does not rule out the possibility of aneuploid embryos due to the male factor.

The prevalence of trisomy 18 (2 to 3%) and 21 (2 to 3%) is similar in blastocysts obtained from own and donor oocytes that were fertilized with semen diagnosed with teratozoospermia. The prevalence does not change in embryos obtained from own oocytes fertilized with sperm diagnosed with normozoospermia.

Likewise, teratozoospermia in our study did not increase the risk of embryos with trisomy 13. In fact, the implantation rate is lower in embryos from own or donated oocytes fertilized with sperm diagnosed with teratozoospermia.

Finally, a prospective study with a larger number of embryos is needed to determine whether the Mexican population, which is characterized by a high prevalence of teratozoospermia, behaved in the same way as the population in this study (Pronatal Clinic).

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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Inconclusive prenatal sex determination in an NIPT due to probable confined placental mosaicism and the importance of amniocentesis.

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ABSTRACT

Non-invasive prenatal diagnosis in maternal blood from placental has become the first-line test for the early detection of chromosomal aneuploidies. Amniocentesis remains the gold standard for the accurate diagnosis of any chromosomal aneuploidy. However, sometimes noninvasive prenatal tests can report inconclusive results which presents a dilemma for decision making. We report a case where fetal sex was inconclusive and confirmation by amniocentesis was performed on a couple.

KEYWORDS: NIPT, amniocentesis, sex determination, mosaicism, inconclusive.

MANUSCRIPT

Introduction

Non-invasive prenatal diagnosis (NIPT) has revolutionized the way prenatal screening can be done in an early, quick, and reliable manner since its first clinical use in 2011. Since its adoption in 2016 for use in all women regardless of age by the American College of Medical Genetics and Genomics, its use has been increasing worldwide as a first-tier method for the detection of chromosomal aneuploidies such as trisomies 13, 18 and 21 and sex chromosome aneuploidies such as monosomy X, XXX, XXY and XYY (1). Importantly, pre-test and post-test genetic counseling should be offered to all couples regardless of their reproductive history. However, as NIPT involves the use of cells originating from the placenta in the maternal bloodstream there is the possibility of inconclusive results due to low fetal fraction or mosaicism. We must remember that the placenta is an organ generated exclusively during pregnancy for the blood supply and nutrients from mother to the fetus and vice versa. That said, placental cells will not always have the same ploidy of chromosomes as the fetus, and this can lead to this type of results due to the presence of probable confined placental mosaicism (CPM), fetal confined mosaicism (FCM) or mixed mosaicism (2,3) in up to 2% of all pregnancies (**Figure**

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1). CPM is usually identified after the first trimester with invasive chorionic villus diagnosis and when mosaicism is detected, amniocentesis is performed to determine if the abnormal cell line is also present in fetal tissues. Amniotic fluid karyotype is usually reported as normal in most cases (72% to 87%) (4,5) In addition, CPM can also be found in cytogenetic studies of the placenta of euploid fetuses (6-8) and is recognized as a relevant source of false-positive results in NIPT (9,10). CPM can be classified into three subtypes (type 1, 2, and 3) depending on where the chromosomal abnormality is in the placenta (**Figure 1**) (Toutain et al., 2018). When the chromosomal abnormality is only found in the cytotrophoblast (and can be found after short-term culture villus examination (STC), it is CPM type 1. If the chromosomal abnormality is only found after a long-term villi culture (LTC), it is restricted to the mesenchymal nucleus of chorionic villi and is classified as type 2. Type 3 is defined as the presence of the abnormality in both the mesenchymal nucleus and the cytotrophoblast and can be found after LTC and STC analysis. As mentioned above, NIPT (and STC-villi) analyzes cytotrophoblast and therefore, NIPT can determine CPM type I and type III (11).

Case presentation

We present a case of a couple Gestation 2 Abortion 1 where both are already of advanced age (38 and 46 years respectively) where there was a previous molar pregnancy. They went to the medical geneticist for advice of a second pregnancy of 14.5 gestational weeks by in vitro fertilization (IVF) and in which they did not want to perform preimplantation genetic diagnosis for aneuploidies (PGT-A), only a NIPT was practiced at week 10.3 of pregnancy where they did not report aneuploidies, nor CNV's of the autosomal and sexual chromosomes, however, fetal sex was reported as "Fetal chromosomal sex not defined by the presence of probable Y chromosome mosaicism" (**Figure 2**). They were provided with genetic counseling in the first instance and opted for amniocentesis to rule out the presence of mosaicism through a karyotype. Mosaicism at this stage is important to understand that it can be confined to placenta, confined to the fetus or mixed (Figure 1) where the result can have important consequences for the fetus and the course of pregnancy if no reported anomaly is verified, so amniocentesis is always necessary and that is why it still remains as the gold standard for a diagnostic method. Amniocentesis in the mother and fetus were performed by a maternal-fetal physician and sent for

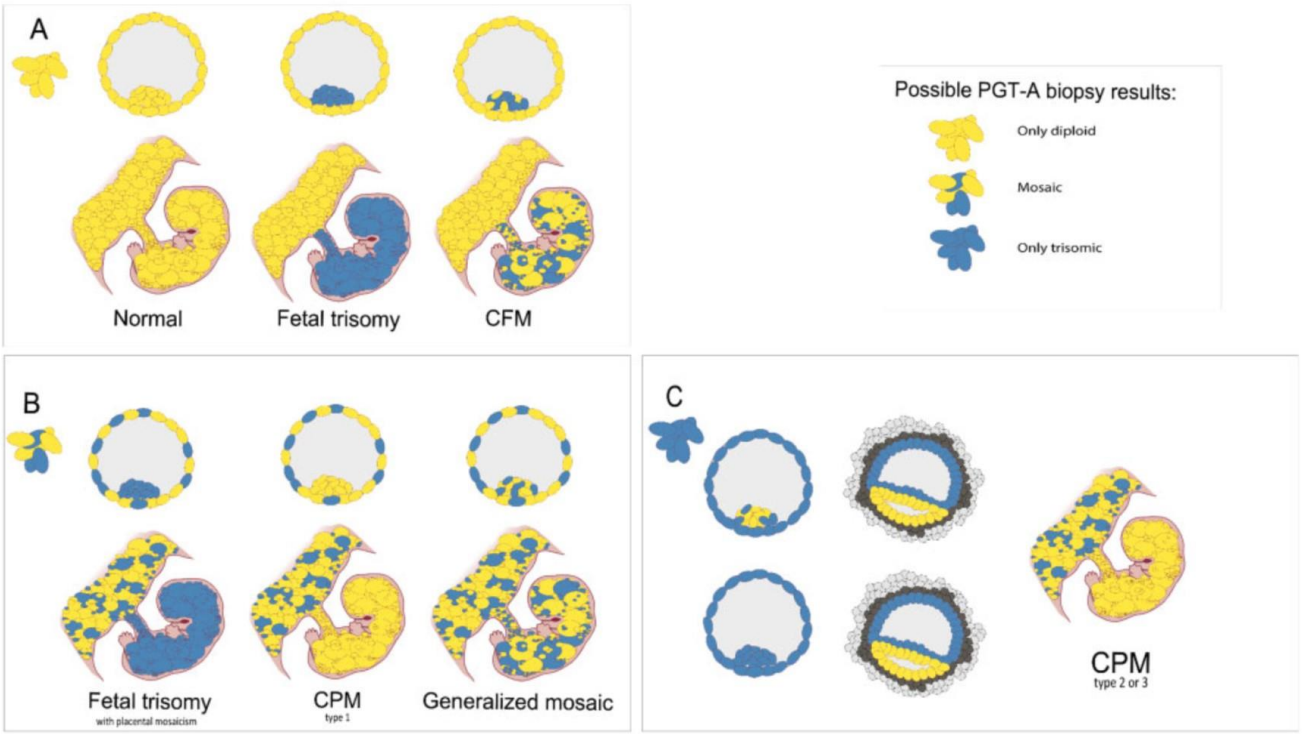

Figure 1. From blastocyst cells to prenatal scenario. (A) Three different scenarios arise if only diploid cells are retrieved through biopsy in blastocyst stage. (B) When both diploid and trisomic cells are biopsied, three different scenarios can also arise. If the mosaicism is only found in the trophectoderm and not within the inner cell mass (ICM), confined placental mosaicim (CPM) type 1 develops. (C) If all biopsied cells appear to be trisomic, even in the ICM, as a result of trisomic rescue, the epiblast will eliminate the trisomic cells and will only consist of diploid cells, thus CPM type 2 or 3 can develop.Taken from Toutain et al., 2018.

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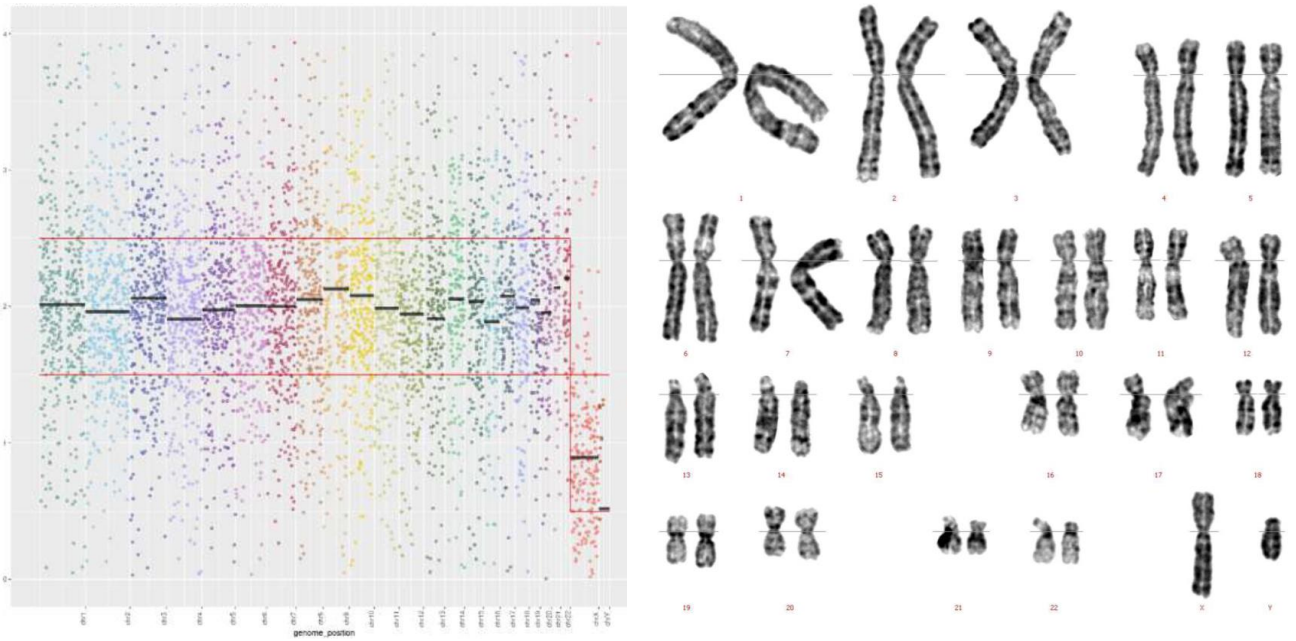
analysis. The karyotype result was reported as 46,XY [30], which corresponds to a male fetus in 30 metaphases (**Figure 2**).



(a) **(b)**

Figure 2. a.Non-invasive prenatal test in maternal blood where an undefined sex chromosome is observed (image courtesy of GD Technologies). B. Karyotype in amniotic fluid where a chromosomal complement of a normal 46, XY male is reported in 30 metaphases (Karyotype courtesy of Genos Médica).

Discussion

Mosaicism is an anomaly that can occur in up to 2% of cases. It is important to note that they can be of 3 types depending on their embryological origin, thus giving 3 types, the CPM, FCM and generalized mosaicism. Regardless of whether it is a PCM, FCM or a generalized mosaicism, it can lead to serious problems in a pregnancy either spontaneously or by assisted reproduction techniques such as aneuploidies that are not compatible with life. In this case report, we demonstrate the importance of performing amniocentesis following a noninvasive prenatal test on maternal blood where fetal sex was reported as inconclusive. Amniocentesis is still the gold standard for determining with 100% accuracy the presence or absence of aneuploidies of autosomes and sex chromosomes when reported as inconclusive by probable mosaicism. In our case it was determined that it was a mosaicism confined to the placenta since only a single cell line was found in the fetal cells of the amniotic fluid.

CONCLUSION

Before a pregnancy performed by IVF, it is advisable to perform PGT-A as it can help drastically to make decisions. When performing a non-invasive prenatal test on maternal blood that is inconclusive, it is imperative to perform amniocentesis to rule out or verify the presence of mosaicism.

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Chorionic bump in ultrasound of the first trimester: Report of a case and bibliographic review.

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ABSTRACT

Background: Chorionic bump involves the gestational sac and can be visualized in first trimester ultrasound. It is described as an irregular convexity that protrudes towards the gestational sac and corresponds to the formation of a hematoma on choriodecidual surface.

Objective: This is a clinical case reported in a second level hospital. An updated search was carried out on the topic of timely diagnosis of chorionic bump.

Case Report: A round echogenic mass protruding from the choriodecidual surface, measuring 17.3 x 16.5 x 19.1mm, was identified during a transvaginal examination at 8 weeks' gestation. Uterine cavity with an irregular Gestational Sac with a mean diameter of 29.9mm with poor decidual reaction, observing the amnion inside without evidence of an embryonic pole and a 2.3mm yolk sac. The pregnancy was not viable.

Discussion: Etiopathogenesis remains unclear. This case demonstrates that a chorionic bump can be considered a poor prognostic finding in ecosonogram.

Conclusion: "Chorionic Bump" is a phenomenon little investigated in ultrasound. The etiology is still unknown. When it is identified, it can be associated with a poor prognostic finding. The most important task for the sonographer is to know how to identify and differentiate it correctly from an early embryonic death.

KEYWORDS: Chorionic bump, hematoma, gestational sac.

MANUSCRIPT

Introduction

The finding of a chorionic bump on ultrasound in the first trimester of pregnancy was initially described by Harris et al as a convex protuberance, with an irregular surface, located on the choriodecidual surface and protruding inward of the gestational sac [(1)]. The reported incidence of chorionic bump ranges from 1.5 to 7 per 1000 pregnancies and is often found

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incidentally [2]. It is a low frequency condition and is not familiar among people who perform obstetric ultrasound examinations. The presence of a chorionic bump is considered a risk factor for a non-viable pregnancy; if it is only an isolated finding, most end up in a live newborn. [3] There is evidence that suggests a guarded prognosis when chorionic protuberance is present, even the risk of spontaneous abortion could be doubled when found on ultrasound in the first trimester [2]. We report the case of a primiparous woman and the sonographic findings related to the presence of a chorionic protuberance in the first trimester.

Clinical case

This is a 29-year-old patient who came for a medical check-up because she reported a delayed menstrual period and a positive urine pregnancy test, in addition to her own pregnancy symptoms. Information is collected from the patient, finding in the anamnesis hereditary family and personal pathological antecedents of no importance to the current condition. In the gynecologic and obstetric history, she has a history of menarche at 10 years of age, with irregular menstrual cycles, and she has interrupted intercourse as a method of family planning. At his visit, he presented only with symptoms of nausea and a history of vomiting of gastric contents on 2 occasions in the previous 24 hours, without referring to transvaginal bleeding. On physical examination, no significant findings were found, the cervix was closed and long, with no transvaginal leaks or discharges. We proceeded to corroborate the diagnosis with an immunological test in pregnancy blood, reporting positive, for which it was decided to perform a vaginal ultrasound. This ultrasound examination concluded: Uterine cavity with an irregular gestational sac with a mean diameter of 29.9mm with poor decidual reaction, observing amnion inside without evidence of an embryonic pole, with a 2.3mm yolk sac. In the chorionic cavity, a hyperechogenic ovoid image is observed, without vascular flow, measuring approximately 17.3 x 16.5 x 19.1mm. Cylindrical cervix without apparent lesions, 43.8mm long, both laterouterine ovaries with anechoic images suggestive of follicles. No image suggestive of free fluid in the posterior fornix. The ultrasonographic study established the Diagnosis: 8 weeks gestation due to gestational sac, anembryonic pregnancy, chorionic protuberance, ovaries with follicles (Figure 1 and Figure 2). For these reasons, definitive surgical treatment with dilation and curettage was decided, sending the tissue collected from the curettage procedure for complementary histopathological study. It was expected that the results would show sections or cystic-based areas in the histopathological study with the possibility of finding fibrin tissue and/or scattered chorionic villi covered by cytotrophoblasts and syncytiotrophoblasts, as has been seen in other publications [10]. However, in our histopathological analysis report, it showed: abundant ovulodecidual remains, various fragments of irregular tissue which together measure 7x4.5x2cm, with areas of a light brown membranous appearance, alternating areas of a hairy, light and dark brown appearance, the tissue presents data of non-specific moderate acute deciduitis with focal microabscess in addition to first trimester chorionic villi and Arias-Stella reaction.

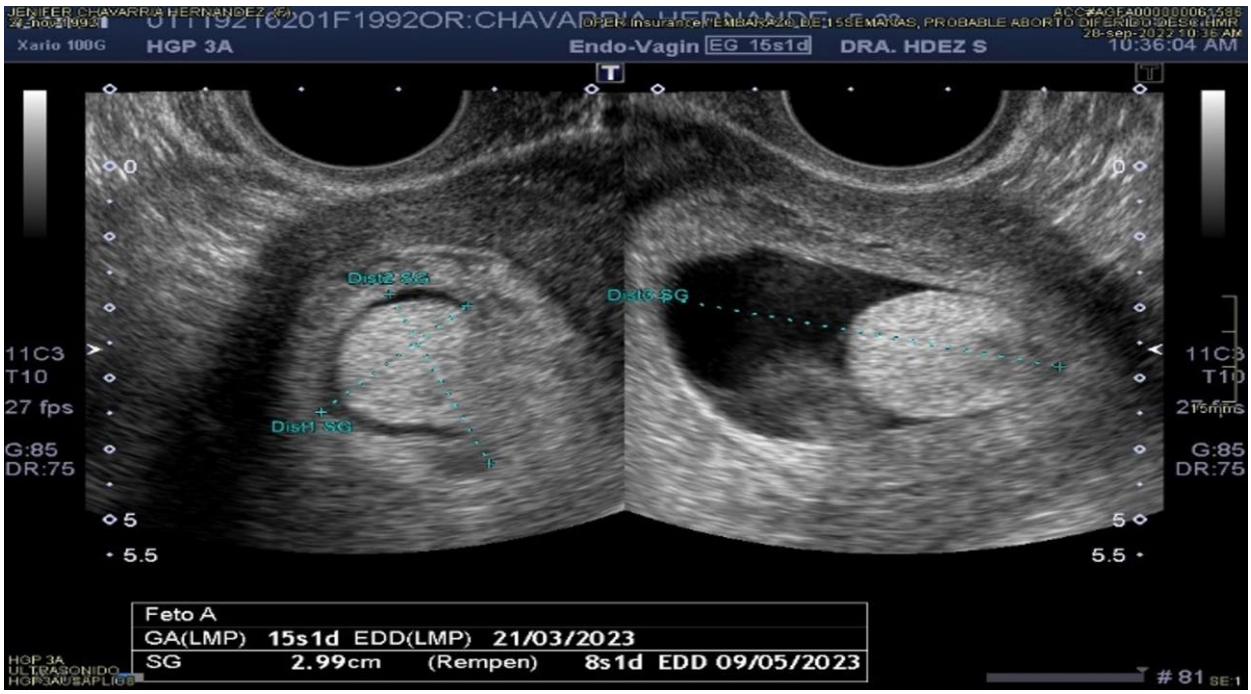

Figure 1: Gestational sac with chorionic bump, in addition to embryo (without cardiac activity) and yolk sac.

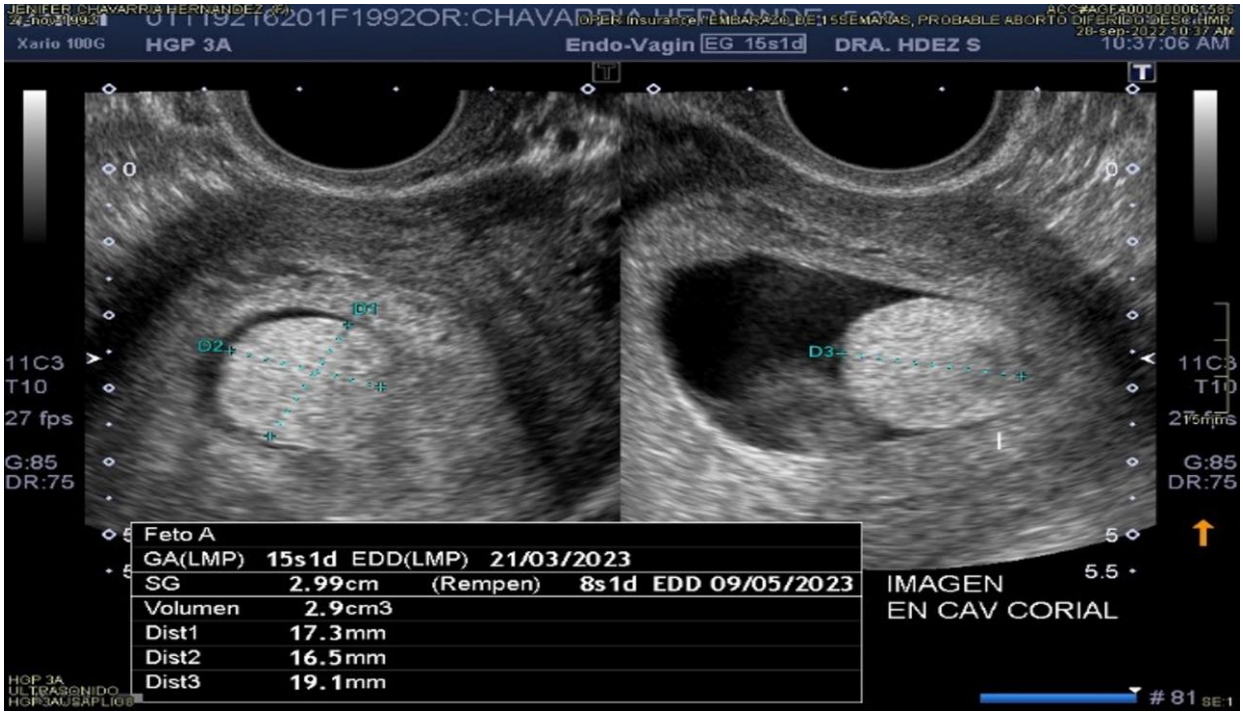

Figure 2. Gestational sac that has a chorionic bump inside.

The patient after her surgical event had no complications, so she went to the recovery service and progressed favorably, so she was discharged 24 hours after the immediate post-surgery with prophylactic antibiotic treatment.

Discussion

The case that we present provides additional evidence on the ultrasonographic finding known as chorionic protuberance. First, we present information on the case and on its ultrasound appearance in the first trimester of pregnancy. To date, most reports have described spontaneous resolution of the chorionic pons in the vast majority of reports. Our case, however, demonstrates that, on some occasions, a Chorionic Protuberance may be present and associated as a Finding of poor obstetric evolutionary prognosis. In this sense, ultrasound is a tool capable of assessing the presence and size of the Chorionic Protuberance, especially if it is detected in a very early pregnancy[4]. Some authors suggest that if normal fetal growth is confirmed in follow-up scans, the risk of pregnancy loss is greatly reduced[5]. In fact, once the pregnancy reaches the end of the first trimester, the pregnancy prognosis in such cases is generally good[5].

Continuing with our case, we describe an unusually poor prognosis early prenatal course associated with an ultrasonographic finding (chorionic protrusion) in the first trimester. This last condition that is described as an image in chorionic cavity hyperechogenic ovoid, without vascular flow that protrudes from the embryonic surface surrounded by a thin membrane and contains areas of echogenic material. There are different authors who believe that this fact is the result of bleeding from the area close to the umbilical cord or the insertion site, secondary to traction and rupture of the fetal vessels that run below the amniotic membrane[6].

In these cases, transvaginal bleeding is not present as in our case, since the amnion firmly contains the clot. Thrombus retraction may produce, over time, the characteristic sonographic features of a subamniotic hematoma with echogenic material retracting at its base surrounded by the layer of the anechoic portion. Therefore, it is suggested that there could have been another pathophysiological mechanism for a subamniotic hematoma, which could have favored the fatal outcome of the embryo in our study. It is for this reason that said hematoma remains covered by the amnion and that it usually does not grow during the rest of the pregnancy. However, adequate surveillance should be carried out in this type of case, since some authors have postulated that large subamniotic hematomas may be associated with fetal growth restriction and/or early embryonic death [6]. An additional associated risk is related to its location; if they are close to the umbilical cord insertion site, and especially if they are large, they can compress the placental vessels and the umbilical cord, leading to adverse fetal outcome[10].

A potential limitation of our study was the fact that the precise umbilical cord insertion site was not determined in the first trimester and the subamniotic hematoma could have developed earlier without being

related to the presence of chorionic protrusion, which could be the cause of the umbilical cord. embryonic death. However, at the time the chorionic bump was detected, there were no other findings on the surface of the placenta to suggest coexisting conditions. As stated above, there are few studies in which only one report of a Chorionic Protuberance progressing to a subamniotic hematoma has been previously described[7, 8].

There have been reported cases in which patients, upon discovering a chorionic protuberance, underwent aneuploidy detection studies of fetal DNA in maternal blood during the first trimester. These studies yielded positive results for trisomies, which were further supported by obtaining a chorionic villus biopsy. The diagnosis was subsequently confirmed through chorionic villus sampling at 12 weeks of gestation. In these instances, the patients opted to terminate the pregnancy. As a result, histopathological analysis of the extracted tissue was conducted, revealing characteristics of immature villi tissue along with hydropic villi and decidual edema[8].

Wax et al. propose that a sonographically isolated chorionic bump could substantially elevate the chances of fetal chromosomal abnormalities in pregnancies with heightened aneuploidy risk. This holds significance for patient counseling. Despite the hematoma hypothesis and the identification of coagulation problems in certain patients, there was no notable link between coagulopathy and successful live births among individuals with a chorionic bump.

However, the documentation available in this case suggests that the chorionic bump may have resulted from a subamniotic hematoma, which may have been the cause of the poor embryonic prognosis. Finally, different authors agree with the fact that chorionic protuberance could be considered a risk factor for non-viability in pregnancy[9]. Likewise, if it is detected in the first trimester, it is ideal to monitor and follow-up by ultrasound, even to look for it in ultrasound scans in the second or third trimester[10].

CONCLUSION

"Chorionic bump" remains a little-researched phenomenon in early pregnancy ultrasound. The etiology is still unknown, but it would represent a small hematoma. Although it is diagnosed infrequently, when first identified, it can be associated as a finding of poor prognosis. The most important task for the sonographer is to know how to correctly identify it and differentiate it from early embryonic death. Other studies need to complement the characteristics found so far in this infrequent ultrasound finding.

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INFORMED CONSENT

Informed consent was obtained from the patient for the publication of her anonymized information. The authors declare that they have followed the protocols of the Mexican Institute of Social Security regarding the publication of the patient's data and that they also have the written consent of the patient for their publication.

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Successful Pregnancy in a Patient with Polycystic Ovary Syndrome Following Lifestyle Modification and Pharmacological Intervention: A Case Report.

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age, often associated with obesity and fertility problems. This case report documents the management of a 32-year-old female patient diagnosed with Polycystic Ovary Syndrome (PCOS), who sought to conceive. Despite having normal hormonal levels, the patient exhibited insulin resistance, a common feature in PCOS. Intervention strategies included pharmacotherapy (metformin, spironolactone, and phentermine) and lifestyle modifications leading to significant weight loss (10.5% of total body weight). The treatments led to an improvement in the patient's metabolic profile, menstrual regularity, and reduction in hyperandrogenic symptoms. By December 2021, the patient had maintained her weight loss and improved metabolic parameters. She successfully conceived in January 2022 and delivered a healthy baby at term in August 2022. This case underscores the importance of weight management and metabolic control in improving fertility outcomes in patients with PCOS.

KEYWORDS: Polycystic Ovary Syndrome; Weight Loss; Insulin Resistance; Lifestyle Modifications; Successful Pregnancy.

MANUSCRIPT

Introduction

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age worldwide. It is characterized by anovulation, hyperandrogenism, and polycystic ovaries. The syndrome has a diverse clinical presentation, with symptoms ranging from menstrual irregularities, hirsutism, acne, and obesity to more severe manifestations such as infertility. PCOS has also been associated with metabolic disorders, including insulin resistance, type 2 diabetes mellitus, dyslipidemia, and cardiovascular disease[1].

The etiology of PCOS is complex and multifactorial, involving genetic, environmental, and lifestyle factors. Several theories have been proposed, with the most widely accepted being that insulin resistance, leading to hyperinsulinemia, plays a key role in the pathogenesis of the syndrome. The resultant

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hyperinsulinemia is believed to stimulate ovarian androgen production, contributing to the hyperandrogenism observed in PCOS. Concurrently, obesity, particularly central obesity, is not only prevalent in PCOS but may further exacerbate insulin resistance and hyperandrogenism, creating a vicious cycle [2-5].

The clinical management of PCOS is challenging due to the heterogeneity of the syndrome. It often requires a tailored, patient-centered approach, addressing both the reproductive and metabolic aspects of the disorder. The cornerstones of management include lifestyle modifications - including diet, physical activity, and weight control - and pharmacological treatments such as oral contraceptives, insulin-sensitizing drugs, and anti-androgens[6].

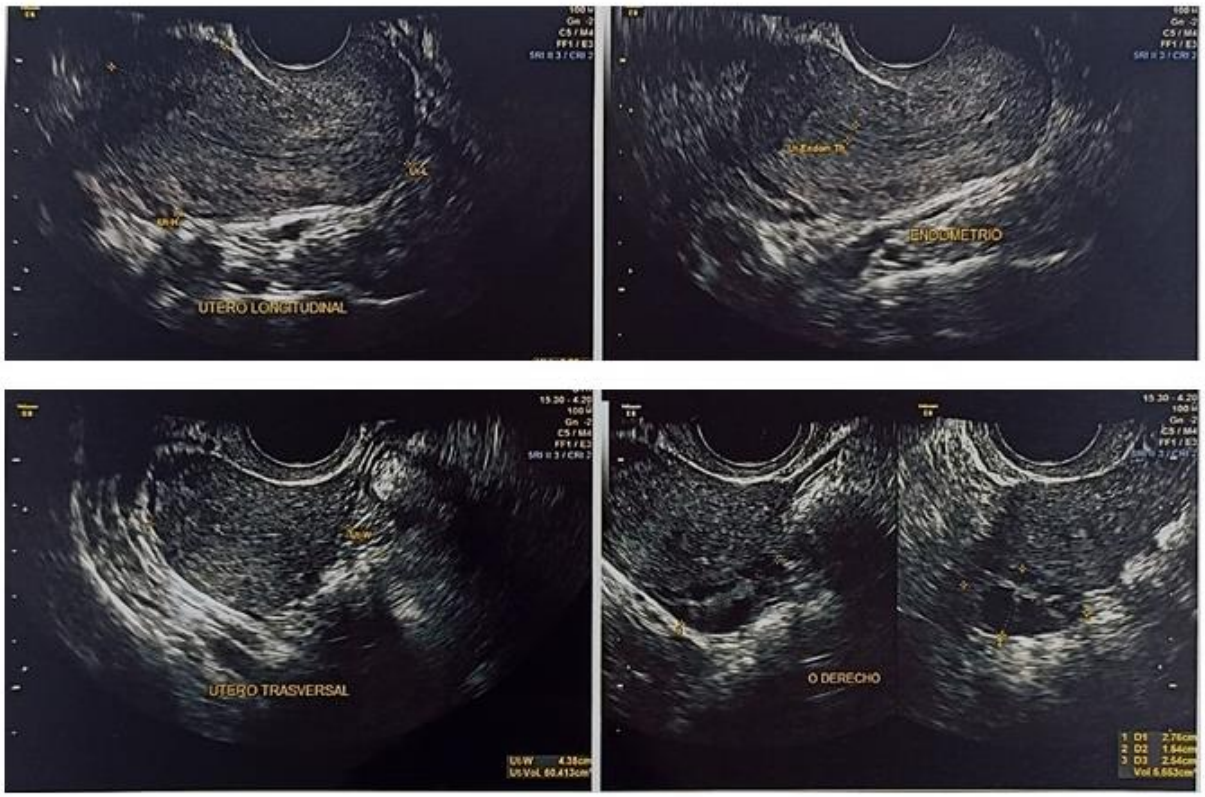
Among the array of therapeutic options, weight reduction has shown promising results, particularly in overweight and obese women with PCOS. Weight loss, as modest as 5-10% of initial body weight, has been associated with improvements in the hormonal and metabolic abnormalities of PCOS, leading to the regularization of menstrual cycles and increased rates of ovulation and fertility. It is believed that weight loss reduces insulin resistance, leading to lower levels of circulating insulin, which in turn decreases androgen production and improves follicular development and ovulation[7].

Pharmacological interventions, such as metformin, an insulin-sensitizing drug, have been widely used in managing PCOS. Metformin improves insulin sensitivity, leading to reductions in insulin levels, and therefore may ameliorate the symptoms of PCOS. Moreover, anti-androgens like spironolactone can effectively manage hirsutism, a frequent and distressing symptom of PCOS. In addition, drugs that promote weight loss, like phentermine, have been used as adjuvants in the management of PCOS[8-9].

In light of the above, the comprehensive management of PCOS in women seeking fertility, such as the one described in this case report, presents a unique opportunity to study the effects of weight reduction, lifestyle modification, and pharmacotherapy on the reproductive and metabolic aspects of PCOS. This patient-centered, multifaceted approach to PCOS could potentially revolutionize how the syndrome is managed, ultimately enhancing the quality of life and reproductive potential of these women.

Case presentation

A 32-year-old female with a primary goal of conception was diagnosed with Polycystic Ovary Syndrome (PCOS), characterized by hyperandrogenism and a cystic pattern on transvaginal ultrasound. She was referred to our weight control service given the well-established link between obesity and PCOS, particularly the role obesity plays in exacerbating PCOS symptoms and fertility issues.



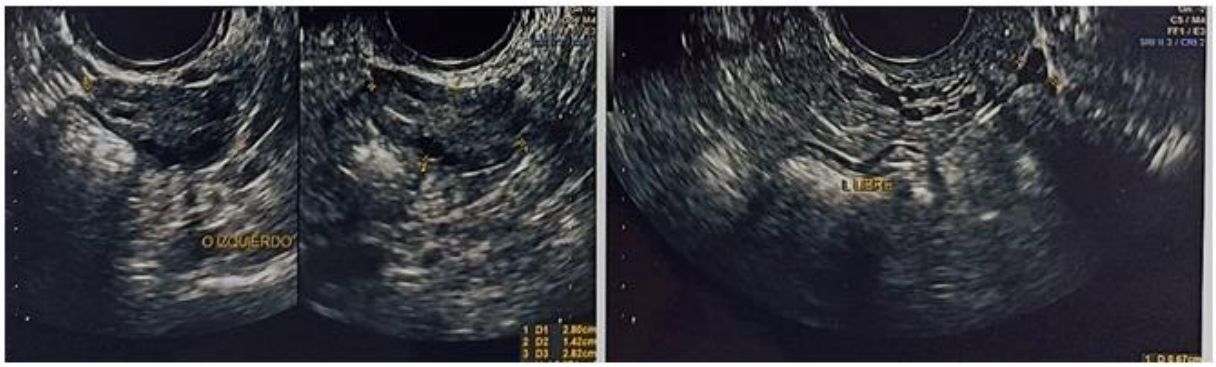


Figure 1. Transvaginal ultrasound images showing bilateral ovarian morphology. The left ovary measures 28x14x28 mm with a volume of 5.8 cc, and the right ovary measures 27x15x25 mm with a volume of 5.6 cc. Both exhibit a follicular pattern and cysts. Homogeneous stromal tissue is observed in both ovaries. The endometrium presents as a Type I, bilaminar, measuring 3.9 mm across both walls.

Figure 1 displays the ultrasound imaging conducted by the Gynecology department, providing visual representation of the patient's polycystic ovarian morphology.

The patient had a family history of systemic arterial hypertension, type 2 diabetes mellitus, and hyperandrogenic PCOS. Her lifestyle was devoid of alcohol, tobacco, and drug use. Her gynecological history included irregular menstrual cycles with amenorrhea, severe cramping, and heavy bleeding. On physical examination, hirsutism was evident, with hair in androgen-dependent areas. Her initial anthropometric measurements indicated Grade I obesity.

Metabolic and hormonal evaluations were performed, revealing insulin resistance and a hormonal pattern compatible with PCOS. The patient was initiated on metformin, spironolactone, and phentermine, with tolerable side effects observed only with phentermine. A comprehensive lifestyle modification plan focusing on diet, physical activity, sleep patterns, and hydration was concurrently implemented. It is relevant to note that a weekly follow-up was maintained with the patient, which likely contributed to improved adherence to the treatment.

Six months following these interventions, she had achieved a significant weight loss of 10.5% from her baseline, with improvement in her anthropometric measures. She also reported regularization of her menstrual cycles and a decrease in hirsutism. Upon achieving the target weight loss, her medication was discontinued, and emphasis was placed on maintaining her healthier lifestyle.

Three months after discontinuation of medication, her laboratory parameters had normalized, and she had maintained her weight loss. In **Table 1**, we present the patient's laboratory results, showing key metabolic and hormonal levels at different stages of the intervention and their response to treatment. Notably, she conceived in the following month. The pregnancy was uneventful, and she delivered a healthy baby girl via caesarean section at 39 weeks gestation.

This case underscores the potential of lifestyle modification in conjunction with pharmacotherapy in managing PCOS, controlling weight, and enhancing fertility.

Discussion

The presented case report provides valuable insights into the management of an atypical case of hyperandrogenic Polycystic Ovary Syndrome (PCOS), highlighting the intricate relationship between hormonal, metabolic, and phenotypic characteristics of the syndrome, and the paramount importance of a patient-centered, multifaceted approach.

PCOS is a heterogeneous disorder with a broad spectrum of manifestations. Our patient, in particular, presented with hirsutism, irregular menstrual cycles, and obesity - classic features of PCOS - but with hormonal levels within the normal range. This atypical presentation underscores the limitations of strictly adhering to a set criterion for diagnosing PCOS, such as the Rotterdam criteria, which emphasizes the presence of hyperandrogenism, ovulatory dysfunction, and polycystic ovaries. It suggests that a more inclusive diagnostic approach may be warranted to capture the full spectrum of PCOS phenotypes, particularly those with normoandrogenic or 'vague' symptoms.

The patient's initial hormonal profile, notably normal, reveals another intriguing aspect of PCOS: the role of insulin resistance. Despite the normal hormonal levels, our patient exhibited significant insulin resistance, as evidenced by the elevated HOMA-IR. This underlines the central role of insulin resistance in the pathogenesis of PCOS, potentially triggering hyperandrogenism and ovulatory dysfunction, independent of overt changes in hormonal levels. Thus, it may be necessary to routinely evaluate insulin resistance in women with PCOS, irrespective of their hormonal status.

	Baseline	Follow-up
Anthropometric Measurements		
Weight (kg)	72	64.5
Height (cm)	150	150
BMI	32	28.5
Classification	Grade I Obesity	Overweight
Body Fat (%)	44	37
Non-fat Mass (kg)	39	38.5
Waist Circumference (cm)	94.4	80
Hip Circumference (cm)	107.5	90
Biochemical Parameters		
Glucose (mg/dL)	87	92
Serum Insulin (UI/dL)	25	10
HOMA-IR	5.2	2.2
Uric Acid (mg/dL)	6.9	4
Cholesterol (mg/dL)	203	180
HDL (mg/dL)	30	36
LDL (mg/dlL	150	100
Triglycerides (mg/dL)	181	132
AST (U/L)	59	9
ALT (U/L)	52	11
GGT (U/L)	66	27
FSH (mUI/L)	6.07	7.29
LH (mUI/L)	1.61	3.93
Prolactin (ng/mL)	19	21.4
Progesterone (ng/mL)	5.67	2.08
Testosterone (ng/mL)	0.22	0.22
Estradiol (pg/mL)	91	113
TSH (Uu/Ml	2.37	1.56
Total T4 (ug/dL)	8.85	12.07
Total T3 (ng/dL)	1.23	0.87

Table 1. Comparison of Anthropometric Measurements and Laboratory Findings at Baseline (March 2021) and at Follow-up (September 2021 and December 2021).

The effectiveness of weight reduction and lifestyle modifications, as demonstrated in our patient, is another crucial point of discussion. The substantial weight loss of 10.5% achieved over six months, along with the implementation of a hypocaloric diet, regular physical activity, and increased water intake, led to improvements in insulin resistance and amelioration of PCOS symptoms. These changes were subsequently followed by the regularization of menstrual cycles, reduction in hirsutism, and, most importantly, a successful pregnancy. This underscores the critical role of weight loss and lifestyle modification in the management of PCOS, especially in overweight or obese patients.

Finally, the use of pharmacological interventions - metformin, spironolactone, and phentermine - provided supportive management for the patient's symptoms and facilitated weight loss. The eventual discontinuation of these medications, without recurrence of symptoms, underlines the effectiveness of lifestyle interventions in managing PCOS.

CONCLUSION

In conclusion, this case study adds to the growing body of evidence supporting the benefits of a comprehensive, individualized approach in managing PCOS. It underscores the potential benefits of focusing not only on hormonal imbalance but also on addressing metabolic issues, specifically insulin resistance and obesity. Future research should continue to explore the complexity and heterogeneity of PCOS to optimize diagnosis and treatment strategies, ultimately improving the reproductive health and quality of life of these patients.

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CONFLICT OF INTEREST

The author declares he has no conflict of interest.

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