Abstract
Objective: To evidence the need for screening fragile X syndrome (FXS) in egg donors in assisted reproduction protocols.
Case report: This is the report of a boy with FXS who inherited the mutated allele from an ovule donated by the mother’s sister through an assisted reproduction protocol. Identifying premutation (PM) carriers of FXS amongst gamete donors isn’t part of the obligatory genetic analysis for donors and is only considered by most of the in vitro fertility societies and guidelines as part of the extension screening tests.
Conclusion: It is cost-effective to do pre-conceptional screening for the PM or full mutation (FM) of the FMR1 gene affected in FXS in every woman undergoing assisted reproductive methods, including gamete donors even without a positive family history of intellectual disabilities. This case supports the need of rethinking the guidelines on the necessary gamete donor screening tests in assisted reproduction protocols.

Keywords: In Vitro Techniques; Fertility; Fragile X Syndrome; Genetic Testing

Introduction
Approximately 9% of couples are infertile and 41% visit assisted reproduction centers in search of alternatives to facilitate or substitute natural fertility and implantation processes (1). About 10% of these couples use egg or sperm donors to achieve pregnancy: Four percent use donated oocytes, 5% sperm and 1% use both (2). Depending on the protocols for each institution, molecular screening tests are performed on donors to identify genetic disease variants with recessive Mendelian inheritance patterns, including the analysis of the FMR1 gene implicated in the fragile X syndrome (FXS). The test for premutation (PM) carriers of FXS is one of the additional screening studies suggested by most of the in vitro fertilization (IVF) societies and guidelines (1, 3–8).

FXS is a genetic X-linked inheritance disease and the first cause of inherited intellectual disability and autism (9). Women with the PM of the FMR1 gene
Fragile X Syndrome and IVF

have a 50% risk in each pregnancy to pass over an abnormal allele to its progeny and therefore them to be affected by FXS. Furthermore, they have an increased risk of developing other phenotypes related to FXS like fragile x linked premature ovarian insufficiency (FXPOI) which is more prevalent in PM carriers with CGG triplet repeats between 59-99 copies (10,11).

The prevalence of PM carriers varies according to the geographic location. Hunter et al. reported a global PM prevalence of 1 per 290 women and 1 per 855 men (12). However, it is important to highlight that depending on the region, prevalence can increase significantly. This is the case of Colombia, a South American country where the highest global prevalence for PM carriers of FXS was reported in a small town. It is 10 to 12 times higher than the prevalence reported by Hunter et al. per 71 men and 1 per 28 women respectively (12, 13).

This is the case report of a child with FXS, the product of an IVF protocol with a family oocyte donor, who inherited the mutated allele for the *FMRI* gene from the egg donated by his aunt on his mother’s side. This case evidences the need to rethink the screening normativity for egg and sperm donors in IVF centers and the importance of including molecular tests that can identify asymptomatic carriers of the PM allele for FXS.

**Case report**

This is the case report of a masculine patient born from the third IVF protocol done on a couple with a history of two previous abortions due to severe endometriosis and two failed IVF protocols with owned oocytes. With this medical history and signs of premature ovarian insufficiency on the female of the couple (menopause by 38 years of age and infertility), the IVF induction for this case was done with the father’s sperm and an egg donated by the mother’s sister, who had two healthy children, male and female, without intellectual disabilities. There was no family history on both sides of autism, intellectual disability or neurodevelopment disorders. Tri-amniotic tri-chorionic triplets were obtained. During prenatal care, multiple ultrasound studies were performed without any relevant findings that suggested congenital anomalies.

The pregnancy was terminated by cesarean section at 28 weeks of gestation due to premature membrane rupture, with extreme premature triplets, two females and one male. One of the girls died after 18 hours. The case reported here spent 53 days in the neonatal intensive care unit due to intracranial hemorrhage.

From an early age, neurodevelopmental delay was evident needing language and occupational therapy. Autism spectrum disorder was suspected, therefore genetic and molecular tests were indicated obtaining a normal karyotype with 46, XY and a RT-PCR for the *FMRI* gene that suggested a full mutation (FM) for FXS. Diagnosis confirmation was done with a Southern blot test which evidenced 300 CGG triplet repetitions, compatible with a FM for FXS.

No screening tests for recessive genetic diseases were performed on the egg donor’s previous IVF protocol. As a result of the FXS diagnosis in the newborn product of this egg donated IVF protocol, a PM carrier status was diagnosed on the egg donor and her sister, the mother of the child. However, only the mother decided to do a Southern blot test to confirm the PM carrier status finding a normal allele and a PM allele. Figure 1 portrays the four family generations of the case reported here. Genotyping results for the number of CGG triplet repeats in the *FMRI* gene on each family member who decided to take the test are described.

By the time the case was evaluated by the genetics and dysmorphology team, the patient was 9 years old, had dysmorphic facies with a long face, wide forehead, winged ears and macroorchidism. Attention deficit and a global neurodevelopmental delay were diagnosed with a cephalic perimeter 75p, with a risk of growth delay (growth for age -1.65 z, BMI for age -0.57z) (14, 15).

The physical exam evidenced a well-mannered patient, as he assists a special teaching school, with apparent adequate social behavior but with learning and language delay; he is only able to pronounce the word “mother” and mumbling. Likewise, he presents a motricity disorder, especially with fine movements with great difficulty for fingers opposition. Parents refer to occasional hetero-aggressive behavior followed up by pediatric psychiatry, no sleep disorders or seizures.

Vitamin C and E supplementation and sertraline were prescribed for learning enhancement and as behavioral modulators respectively with adequate results, especially in terms of aggressive behavior control.

**Discussion**

This is the case of a 9-year-old male with a FM of the *FMRI* gene involved in FXS, a product of a pregnancy obtained by IVF protocol.
The genetic mutation was passed on by the egg donor who was the mother’s sister who by the time of the IVF didn’t know about her PM carrier status and had no personal or family history of intellectual disability.

The American College of Obstetricians and Gynecologists Committee on Genetics (3), the American Society for Reproductive Medicine (16) and the American College of Obstetricians and Gynecologists since 2017 (17) recommend screening for FMR1 gene PM only in women with a family history of FXS, intellectual disability, neurodevelopment delays with unknown cause or women with ovarian insufficiency before 40 years of age. PM carrier screening for genetic disorders is used to identify the presence of pathogenic allelic variations in people with no evident pathologic phenotype. In potential gamete donors, these tests are used to determine the genetic compatibility with the counterpart biologic parent in order to avoid obtaining newborns with recessive genetic diseases. Most carrier screening protocols recommend the active search of high frequency recessive autosomal diseases like cystic fibrosis, spinal muscular atrophy and hemoglobinopathies. Nevertheless, the molecular test for FXS is not considered by European or American guidelines as part of the initial protocols for gamete donors (1, 3–8). The egg donor in this case, who was also a family member of the patient, didn’t comply with any of the aforementioned criteria for screening, therefore no molecular study for FXS was indicated.

There are at least two previous reports of PM allele inheritance for the FMR1 gene from gamete donors who didn’t know about their PM carrier status by the time of donation. The first was an anonymous sperm donor (18) and the second an anonymous egg donor (19). PM carriers in the general population are fairly prevalent, making evident the need to update the current guidelines for gamete donor screening protocols, specifically on the criteria to consider molecular screening tests for FXS.

There are several papers on the frequency of PM carriers in groups of women who comply with the current criteria for prenatal screening of FMR1 gene variations. In women with a family history of intellectual disabilities or neurodevelopmental disorders, the prevalence of FMR1 gene mutation has been as common as 1 per 86 (20) to 1 per 128 women (21–23). While in women without this family background the prevalence for PM is 1 per 157 (23) to 1 per 788 (24) (Table 1).

Due to the high prevalence of PM women carriers without a relevant family history, some scientific societies have updated their guidelines to consider PM carrier risk for FXS. The Spanish Fertility Society the Spanish Association of Andrology, the Spanish Association of Medical Biopathology and Laboratory Medicine, the Association for the Study of Reproductive Biology and the Spanish Association of Human Genetics (30), differing from most obstetrics, gynecology and genetics guidelines for the United States or Europe (1,3–8,31,32); have included in their protocols the detection of the number of CGG triplet repeats present in the FMR1 gene, without taking into account family history, as part of the mandatory screening tests to potential egg donors (1,3–8,30–32).
Table 1: Frequency of permutation (PM) women carriers of fragile X syndrome (FXS) in different population groups and countries around the world. (20, 21, 23–29). USA (United States of America).

| Frequency of PM women carriers | Studied population                                      | Country              |
|--------------------------------|---------------------------------------------------------|----------------------|
| 1 in 259                       | Women in general population                              | Canada               |
| 1 in 777                       | Pregnant women                                           | Chinese Han population|
| 1 in 788                       | Pregnant women                                           | South Korea          |
| 1 in 257                       | Pregnant women without FXS risk criteria                 | USA                  |
| 1 in 86                        | Pregnant women with FXS risk criteria                    | USA                  |
| 1 in 178                       | Patients with suspicion of being PM carrier              | USA                  |
| 1 in 246                       | Pregnant women                                           | Finland              |
| 1 in 128                       | Women with family history of intellectual disability or neurodevelopmental disorders | Israel               |
| 1 in 157                       | Women without family history of intellectual disability or neurodevelopmental disorders | Israel               |

Screening for FXS in egg donors is necessary as PM women carriers can have a normal phenotype, especially during reproductive age. They don’t have any distinctive physical features or intellectual disabilities which allow the identification of their PM carrier status. Furthermore, due to the decreasing number of extracted eggs and the need for higher doses of gonadotropin hormone, compared to women without the PM or even with the FM; PM carriers have a lower success rate in IVF protocols (33, 34).

PM carriers can pass on a mutated FMR1 gene allele in 50% of cases. There is a possibility that the PM allele can expand from a PM to a FM and produce an embryo with FXS. The risk of expansion increases as the number of CGG triplet repeats increase, women with more than 100 CGG triplets will pass on an expanded allele in 100% of cases. Likewise, a lower number of AGG interruptions and older maternal age are risk factors for allele expansion to FM.

The egg receptor for this case had two previous failed attempts of IVF with her own eggs; she was the donor’s sister and was also a PM carrier. This enhances the need for young women with PM for FXS, even without a reproductive desire, to undergo ovarian reserve tests and in case of a decreased reserve be offered fertility preservation alternatives (33, 34).

The biggest question for health systems is whether it is cost-effective to screen every pregnant woman or woman undergoing IVF processes for the PM of FXS. The answer to this question depends on the frequency of PM carriers in the population being analyzed. Musci et al. with a prevalence of 1 per 303 with the PM, concluded that it is actually economically beneficial to identify PM carriers in pregnant women (35). The prevalence reported by Musci et al. is similar to the one reported for North America and Europe (20, 21, 26, 27) and even lower than the one reported in Israel (23, 28) (Table 1). Zhang et al., created a cost-effective model to screen every adult between 18 and 25 years of age in Australia for cystic fibrosis, spinal muscular atrophy and FXS; finally concluding that a massive screening could decrease the number of combined cases for these three diseases by 25%. Compared to a selective screening of people with positive family history, this broad screening model turned out to be better, economically speaking (36). Communities with lower prevalence of PM carriers, like Asian countries, have also conducted a cost-effectiveness analysis for the screening of FXS. A retrospective study done in Taiwan identified 26 women with the PM from 20,199 women screened for the number of CGG triplet repeats (25), with a reported prevalence of 1 per 777 women with the PM. As they compared the prenatal and pre-conceptional diagnosis costs, this study concluded that screening for PM carriers could be cost-effective (25).

One of the main reasons against doing a general population screening for the identification of PM carriers of FXS is the comprehension of the inheritance pattern and the FXS phenotypes (1,3–8,31,32). Johansen et al. analyzed reproductive decisions of 30 women diagnosed as PM carriers who didn’t have any criteria to do a molecular test screening for this genetic disease; versus 92 women who were diagnosed as PM carriers during routine screening due to positive family history or any of the criteria of the current guidelines for pre-conceptional or prenatal diagnosis of the PM carrier status of FXS. The researchers didn’t find any significant statistical differences in the decisions made by couples after the post-test genetic counseling. 74% of couples meeting...
the criteria for screening versus 55% of couples who didn’t meet the standardized requirements for screening, decided to take action in order to reduce the risk of having a genetically affected child. Furthermore, there weren’t any differences when the diagnosis was made as part of the prenatal studies; therefore, screening for PM carriers of FXS should be open to the general population and not only the group meeting the current criteria for screening (37).

The high prevalence of PM carriers for FXS in the general population and therefore the increased risk for FM expansion on to the next generation associated with an increased rate of IVF; pre-conceptional screening for the PM or FM of the FMR1 gene affected in FXS should be considered in every woman undergoing assisted reproductive methods, including gamete donors even without a positive family history of intellectual disabilities (3, 35, 37–39).

Conclusion
It is cost-effective to do pre-conceptional screening for the PM or full mutation (FM) of the FMR1 gene affected in FXS in every woman undergoing assisted reproductive methods, including gamete donors even without a positive family history of intellectual disabilities. This case supports the need of rethinking the guidelines on the necessary gamete donor screening tests in assisted reproduction protocols.

Conflict of Interests
Authors have no conflict of interests.

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