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Three Male Siblings Undergoing Multiple IVF Failures Where a PLCZ1 Mutation Finally Revealed After a WES Analysis. A Three Couple's Odyssey due to a Genetic Cause Ended Using Donor Sperm Eventually: A Family Case Report

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Abstract

We present a case involving three brothers, all experiencing either normo- or oligospermia, who underwent several *In-Vitro* Fertilization (IVF) procedures without achieving a successful live birth. Each couple struggled with male or unexplained primary infertility for 10-15 years. Upon genetic testing, the second brother underwent Whole Exome Sequencing (WES), which revealed a mutation in the phospholipase C zeta 1 (PLCζ1) gene. This discovery introduced a new dimension to their infertility struggle, shedding light on the underlying issue hindering their ability to conceive.

The PLCζ1 gene, situated on chromosome 12, governs the production of the PLCζ1 enzyme. This enzyme primarily operates within the head of spermatozoa, playing a crucial role in regulating calcium signaling during fertilization. PLCζ1 facilitates the generation of inositol trisphosphate (IP3) within sperm cells, initiating a cascade of calcium ion (Ca^{2+}) release events vital for fertilization, including egg activation and the fusion of sperm and egg nuclei.

With the knowledge of their genetic condition, all three brothers opted for the use of donor sperm. Consequently, two of them have since become parents, while the third is currently undergoing a pregnancy. This case underlies the importance of WES analysis in diagnosing cases of prolonged male and/or female infertility and repeated IVF failures.

Keywords: PLCζ1 mutation • Male infertility • Total Fertilization Failure (TTF) • WES analysis

Introduction

For decades, majority of fertility specialists have predominantly focused on female infertility, inadvertently neglecting the male aspect, despite the transformative impact of Intracytoplasmic Sperm Injection (ICSI) in treating males with Oligoasthenoteratospermia (OAT). Consequently, the male factor has been marginalized and understudied.

Male infertility has various underlying causes, broadly categorized into endocrine disorders, sperm transport disorders, primary testicular issues, and idiopathic factors [1]. While semen analysis remains fundamental in assessing male infertility, advancements in diagnostic tests have emerged to enhance the evaluation of sperm quality and function, thereby improving diagnosis and management [2].

Fertilization encompasses a complex sequence of events culminating in the fusion of sperm and egg nuclei, resulting in zygote formation. Key steps include sperm penetration of the zona pellucida and subsequent binding to and fusion with the oocyte's plasma membrane. Molecular interactions and signaling events, facilitated by specific factors known as Sperm Oocyte Activating Factors (SOAF) Amdani SN, et al. [3] are crucial in this process. Poor or complete fertilization failure following Intracytoplasmic Sperm Injection (ICSI) poses significant challenges for both patients and healthcare providers, with deficient oocyte activation often identified as the primary cause [4].

Despite the overall success of ICSI, a small percentage (1-3%) of cases encounters poor (PFF) or Total Fertilization Failure (TFF). TFF refers to the situation where all mature oocytes retrieved from the female fail to fertilize within a single cycle [5]. Oocyte Activation Deficiency (OAD) stands out as the leading cause of TFF in ICSI cycles, indicating a failure of oocytes to undergo further maturation and complete fertilization post-sperm fusion. Recent research has linked OAD to various abnormalities in PLCζ1 observed in male patients who have experienced previous failed IVF or ICSI cycles [4].

Phospholipase C zeta 1 (PLCζ1), also referred to as PLCZ1, serves as a vital enzyme in the fertilization process, particularly in initiating intracellular calcium (Ca^{2+}) oscillations within the egg upon sperm entry, which are essential for embryonic development commencement. Predominantly located in sperm cells, PLCζ1 plays a pivotal role during fertilization when a sperm penetrates the egg's membrane, releasing PLCζ1 into the egg cytoplasm. Subsequently, PLCζ1 catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into two secondary messengers: Inositol 1,4,5-trisphosphate (IP3) and Diacylglycerol (DAG) [6]. IP3 production by PLCζ1 prompts the release of calcium ions (Ca^{2+}) from the Endoplasmic Reticulum (ER) within the egg, initiating crucial calcium oscillations necessary for egg activation, early embryonic development, prevention of polyspermy, and facilitation of meiosis completion in the egg [7].

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The human PLCZ1 gene comprises 15 exons and is situated on chromosome 12. Studies in current literature reveal various localization patterns of PLCZ1 in sperm, with consensus pointing towards the equatorial region as the most valid pattern. Expression levels and localization patterns of PLCZ1 exhibit significant variability, observed in both fertile donors and infertile patients. Notably, patients with globozoospermia display absent or abnormal PLCZ1 levels in their sperm, characterized by punctuated localization patterns [7]. One hypothesis suggests that PLCZ1 levels may decrease during spermatid elongation; however, further investigation is required to delineate PLCZ1 localization patterns within the human testes [4].

Mutations or defects in the PLCZ1 gene within sperm can disrupt egg activation and lead to fertilization failure. In cases where fertilization issues are suspected to stem from a sperm factor, such as in couples with unexplained fertility problems, assessing the presence and function of PLCζ1 in sperm may be warranted [8].

Therapeutic intervention such as Artificial Oocyte Activation (AOA) is proposed in the literature to address the absence of Ca^{2+} oscillations resulting from mutations in the PLCZ1 gene. AOA aims to rescue oocytes from fertilization failure and enhance the two Pronuclei (2PN) rate. However, statistically, only 25% of patients with bi-allelic PLCZ1 mutations undergoing ICSI achieve successful childbirth, underscoring the importance of PLCZ1 in embryonic development [9].

Case Presentation

Couple comprising the eldest brother

In 2019, a couple struggling with infertility, having undergone unsuccessful IVF attempts since 2004 (a 15-year period of infertility), sought assistance at our clinic. Both partners exhibited normal karyotypes and had endured six failed IVF cycles. During the initial four IVF cycles, embryologists observed a poor fertilization rate, with all embryos ceasing growth by day 3 of culture. Despite transferring day 2 embryos in the third and fourth attempts, no pregnancy achieved. In 2010, they pursued the fifth and sixth cycles using donor sperm, fertilizing half of the wife's oocytes with her husband's sperm and the other half with donor sperm. However, embryos derived from the husband's sperm failed to fertilize or developmentally arrested by day 3. Even embryos generated using donor sperm in the sixth cycle failed to progress to the blastocyst stage, complicating diagnosis and causing profound disappointment.

Following a decade-long hiatus, the couple returned to our clinic in 2020. We recommended a final attempt using the wife's oocytes, now aged 38, with Preimplantation Genetic Testing for Aneuploidy (PGTA) and calcium ionophore oocyte activation, utilizing a 50-50% combination of the husband's and donor sperm. Despite these efforts, no euploid embryos were obtained from either the donor (2 blastocysts) or the husband's sperm (1 poor-quality blastocyst) in 2020. Consequently, in 2021, we pursued embryo donation with both oocytes and sperm sourced from donors. Following the transfer of two blastocysts, a twin pregnancy ensued, culminating in the birth of two live-born infants.

During this period, the mutation discovered in the second brother prompted the first-born to undergo whole exome sequencing analysis, which revealed the same mutation in the PLCZ1 gene.

Couple comprising the second brother

In the meantime, the younger brother of the previous couple had been grappling with similar infertility challenges since 2010. Like his sibling, this couple also endured multiple unsuccessful IVF attempts. Initially, they underwent two cycles of controlled ovarian stimulation and IVF, during which either no fertilization occurred, or all embryos ceased by day 3 of development. Subsequently, they pursued several natural cycle IVF attempts, all yielding negative results. Amidst these efforts and numerous examinations, a geneticist observed a pattern of failed fertilization and blastocyst formation in both brothers, prompting a recommendation for Whole Exome Sequencing (WES) for the male partner in this couple. The WES analysis conducted in 2019 unveiled two distinct mutations in the PLCZ1 gene.

The first mutation identified was a heterozygous missense mutation, specifically a histidine to leucine substitution at position 233 (PLCZH233L) c.698A>T (p.His233Leu). This mutation, described as damaging and disease-causing, can be inherited through the maternal lineage. The substitution of histidine, a polar and hydrophilic amino acid, with leucine, a non-polar hydrophobic amino acid, appears to disrupt local protein folding interactions, leading to diminished activation rates [8,10]. The second mutation detected was a nonsense mutation, resulting in the premature termination of protein translation due to the production of a stop codon. While this mutation is categorized as potentially disease-causing, limited information is available from existing databases and literature, aside from our current case.

Upon learning that their elder brother had successfully opted for donor sperm and achieved parenthood, this couple sought guidance from our clinic. Following consultation, we recommended proceeding with donor sperm and the wife's oocytes. Subsequently, in 2022, they underwent controlled ovarian stimulation, resulting in the retrieval of 19 oocytes. These oocytes were fertilized with donor sperm and 3 embryos developed into blastocysts. One of the blastocysts was transferred, resulting in the birth of a healthy live-born infant in 2023.

Couple comprising the third brother

The younger brother also encountered infertility issues starting from 2011, compounded by his wife's low Anti-Müllerian Hormone (AMH) levels. Despite undergoing three IVF trials, they were unable to achieve a pregnancy. In 2020, they visited our clinic with the intention of proceeding with donor sperm, particularly since the mutation in his brother had been identified, but still utilizing wife's oocytes. However, given her significantly diminished ovarian reserve (1.07 ng/ml), natural cycle IVF was proposed. Unfortunately, after three attempts, no pregnancy ensued.

Subsequently, in 2023, they opted for double gamete donation. During the initial embryo donation cycle, two blastocysts were transferred, resulting in a biochemical pregnancy. However, with the second embryo donation cycle, they achieved a successful pregnancy, and she is currently at 9 weeks of gestation.

Discussion

The current family case with 3 brothers facing cumulatively more than 35 years of infertility and several failed IVF attempts signifies the role of genetic investigation in certain cases. Moreover, the role of WES analysis in both partners of couples with long term infertility, poor fertilization, poor blastocyst formation and Repeated IVF Failures (RIF), might look promising.

In our case we report three infertile brothers presenting oocyte activation failure who were carriers of the same mutation in PLCZ1 gene. The mutation led to an almost complete disappearance of the protein in the patients' sperm. Increasing evidence suggests that dysfunctional forms of the oocyte activation factor PLCZ may underlie certain types of male factor infertility [4,10].

Nevertheless, it was unclear how this heterozygous mutation could cause infertility, resulting in compound heterozygosity, which reinforced the link between PLCZ1, OAF and infertility. Importantly, this mutation was not associated with teratozoospermia, and the numbers of normal sperm were well above the lower accepted reference values. Moreover, the sperm of all 3 brothers showed only slightly reduced DNA quality, ruling this out as the cause of OAF. Altogether, these results indicate that PLCZ1 plays a direct and primary role in the activation of mammalian oocytes.

Interestingly, only one brother managed to achieve a livebirth with his wife eggs. The other two brothers due to the prolonged years of infertility and lowering ovarian reserve of their wives managed to become pregnant but only after using donor oocytes as well. This is sad, as probably if the genetic diagnosis had been made much earlier, these women might have avoided the use of donor eggs.

Conclusion

In summary, we suggest that more research need be done on this gene and the mutations that exist. Because of the heterogeneity of the mutations, each couple needs individual medical care, which is appropriate for the mutation that a man carries. We did not find out the exact mechanism that linked these two mutations. Although calcium ionophore activation is recommended, and sometimes it works for some couples who carry common mutations in PLCZ1 gene Wang F, et al. and Mu J, et al. definitely is not suitable for everyone. In our three cases, AOA did not work and the only solution was using donor sperm that eventually resulted in the livebirth of two healthy babies and one ongoing pregnancy.

Acknowledgment

None.

Conflict of Interest

None.

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