Phospholipase C zeta and oocyte activation defects: moving toward the objective identification of patients eligible for artificial oocyte activation

Oocyte activation deficiency (OAD) seems to be the main factor contributing to poor or total fertilization failure in assisted reproductive technology cycles. The phenomenon occurs in ~1%–5% of intracytoplasmic sperm injection (ICSI) cycles and usually recurs. Under normal conditions, oocyte activation involves morphologic and biochemical changes that allow the oocyte to complete meiosis and initiate embryogenesis. The process is triggered by sperm factors and is mostly dependent on the increase in cytosolic free Ca^{2+} level, which occurs as a prolonged sequence of repetitive Ca^{2+} transients, known as Ca^{2+} oscillations.

Some soluble sperm-derived molecules, including phospholipase C zeta (PLCζ), can diffuse into the oocyte cytosol and promote Ca^{2+} oscillations via the inositol 1,4,5-trisphosphate (InsP3) signaling pathway. PLCζ is a testis-specific PLC present as an RNA in spermatids and only found as a protein in mature sperm. Current data indicate that OAD relates to a disrupted PLCζ activity caused by abrogated, reduced, or aberrant forms of PLCζ. Such deficiencies have been associated with both evident (e.g., globozoospermia) and subtle (eg., mutant forms of the protein found in normozoospermic infertile men exhibiting poor oocyte-activating ability) sperm abnormalities.

The assessment of PLCζ activity has gained increased interest owing to its potential clinical value for providing laboratory evidence of PLCζ deficiency and selecting candidates for artificial oocyte activation (AOA). However, the existing diagnostic methods have yielded conflicting results regarding PLCζ localization pattern and quantification level. In this issue of *Fertility and Sterility*, Meng et al. provide further evidence supporting the clinical utility of PLCζ testing to guide AOA (1). Using an in-house immunofluorescence-staining PLCζ assay, the authors screened infertile couples suspected of having OAD, including those with a history of total fertilization failure, low fertilization rate (<50%), and recurrent fertilization failure in IVF/ICSI cycles.

After PLCζ quantification, patients were divided according to the mean levels of PLCζ in sperm and the proportion of sperm exhibiting PLCζ. The data were then compared with that of a control group composed of fertile men. Meng et al. found that ~80% of couples with a suspected OAD had either reduced PLCζ, namely, sperm with low PLCζ levels or low proportions of sperm exhibiting PLCζ, or deficient PLCζ, when both of the above defects were combined. While their findings confirm previous observations of a strong association between PLCζ and OAD, the authors added to the literature by investigating the effect of AOA in patients with the most severe PLCζ defect, namely, PLCζ deficiency; these patients composed 40% of their patient population. The subset of patients who agreed to undergo ICSI with the use of AOA (AOA-ICSI) achieved significantly higher fertilization rates than those recorded in previous cycles without AOA.

Interestingly, although this study showed that PLCζ deficiencies relate to abnormal sperm morphology, e.g., globozoospermia, three of five patients undergoing AOA-ICSI had semen parameters within normal ranges, thus suggesting that conventional semen analysis alone is unable to determine who might benefit of PLCζ testing or AOA. The authors established cutoff values based on the mean PLCζ level in sperm and the proportion of sperm containing PLCζ to objectively identify patients with PLCζ-related OAD who could potentially benefit from AOA-ICSI.

We commend the authors for conducting such an elegant study and discuss some of its limitations below. First, most patients (~60%) had the less severe form of PLCζ defect, i.e., reduced PLCζ, but unfortunately, none of those patients underwent AOA-ICSI. Although fertilization and live births were obtained without the use of AOA in three couples of their cohort of patients with reduced-PLCζ, their findings could be explained by the random pickup of sperm containing adequate PLCζ. Thus, we believe it is also essential to investigate the effect of AOA-ICSI in patients with reduced PLCζ to better understand the potential value of PLCζ testing as guidance for AOA.

Second, although the assay developed by Meng et al. (1) provides laboratory evidence of PLCζ abnormalities, many technical questions concerning its diagnostic accuracy remain unanswered. For example, it is not clear if the number of cells assayed is large enough to provide an accurate estimation of the PLCζ status. It is also uncertain whether ejaculatory abstinence, and particularly sperm viability, which was not controlled for, affect PLCζ results. The latter is critical because dead sperm do not usually exhibit the characteristic staining pattern. In addition, the assay has limitations regarding patient eligibility: individuals with low sperm counts and those who had sperm surgically retrieved are not eligible for testing. Thus, even with further refinements, the PLCζ testing described by Meng et al. might not be informative in patients with nonobstructive azoospermia—with testicular sperm available for ICSI—because PLCζ expression only initiates at the final germ cell differentiation stages. Despite understanding the complexity of validating a cell-based fluorescence diagnostic method, we think the above issues have to be discussed further before the implementation of PLCζ testing in clinical settings.

The study by Meng et al. (1) also raises intriguing clinical questions. For example, why did AOA-ICSI fail to improve fertilization in some PLCζ-deficient patients? An exemplary PLCζ-deficient case involving a globozoospermic patient illustrates this scenario; in this case, the fertilization rates remained virtually unchanged after AOA-ICSI. In their study, AOA was carried out with the use of Ca^{2+} ionophore. Unlike the typical physiologic Ca^{2+} oscillations triggered by PLCζ, Ca^{2+} ionophores are synthetic chemicals that induce a single large Ca^{2+} transient. Concerns exist that Ca^{2+} ionophores might be insufficient to promote adequate oocyte activation.

In contrast, the latest research has focused on the use of recombinant PLCζ as a physiologic alternative to overcome OAD. Preliminary results have shown that AOA with the use of recombinant PLCζ can rescue failed oocyte activations; however, its safety and potential adverse effects for embryogenesis and resulting offspring are unknown (2). Nevertheless, there is a remarkable variation in patient response to oocyte activation with recombinant PLCζ, as measured by Ca^{2+} oscillations, thus suggesting that PLCζ might not be the only critical factor for oocyte activation. In fact, transgenic knockout mice for PLCζ, generated with the use of CRISPR/Cas technology, can produce viable offspring, albeit with low efficiency (3). It is, therefore, plausible that other sperm-associated proteins act in synergy with PLCζ to trigger fertilization and early embryogenesis events. Moreover, oocyte factors, including nuclear and cytoplasmic maturation events, may also play a role during the activation process.

While waiting for PLCζ assay refinements and further validation, as well as more clinical data concerning the use of recombinant PLCζ, we believe AOA with Ca^{2+} ionophore could be considered in patients with a history of repeated (total/low) fertilization failure regardless of whether a PLCζ deficiency has been identified or assessed. Along these lines, AOA-ICSI could be also considered in patients with nonobstructive azoospermia owing to the suboptimal fertilization rates after testicular sperm injections. (4). On one hand, the current evidence concerning AOA safety in humans is reassuring, but data is minimal. On the other hand, mammalian studies suggest that the monotonic Ca^{2+} release triggered by Ca^{2+} ionophore might have epigenetic, mutagenic, and cytotoxic effects on embryogenesis (5). Naturally, patients should be fully informed about the advantages and risks of AOA-ICSI, and the need for preimplantation genetic testing for aneuploidy should be discussed.

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REFERENCES

1. Meng X, Melo P, Jones C, Ross C, Mounce G, Turner K, et al. The use of phospholipase C zeta (PLCζ) analysis to identify candidates for artificial oocyte activation (AOA): a case series of clinical pregnancies and a proposed algorithm for patient management. Fertil Steril 2020;114:163–74.
2. Nomikos M, Yu Y, Elgmati K, Theodoridou M, Campbell K, Vassilakopoulou V, et al. Phospholipase Cζ rescues failed oocyte activation in a prototype of male factor infertility. Fertil Steril 2013;99:76–85.
3. Hachem A, Godwin J, Ruas M, Lee HC, Ferrer Buitrago M, Ardestani G, et al. PLCζ is the physiological trigger of the Ca^{2+} oscillations that induce embryogenesis in mammals but conception can occur in its absence. Development 2017;144:2914–24.
4. Esteves SC, Carvalho JF, Bento FC, Santos JA. Novel predictive model to estimate the number of mature oocytes required for obtaining at least one euploid blastocyst for transfer in couples undergoing in vitro fertilization/intracytoplasmic sperm injection: the ART calculator. Front Endocrinol (Lausanne) 2019;28:10–99.
5. Ozil JP, Banrezes B, Toth S, Pan H, Schultz RM. Ca^{2+} oscillatory pattern in fertilized mouse eggs affects gene expression and development to term. Dev Biol 2006;300:534–44.