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COMMENTARY

Artificial oocyte activation: evidence for clinical readiness

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Abstract Artificial oocyte activation using Ca^{2+}ionophores or similar compounds is a widely applied technique in IVF laboratories. This is all the more interesting as most of the agents aiming for intracellular Ca^{2+} increase do not result in physiological Ca^{2+} oscillations but much rather cause a single Ca^{2+} transient. Two observations from mammals may explain why a rather non-physiological single Ca^{2+} peak caused by ionophores is sufficient to rescue cycles showing severe male factor infertility, deficient oocyte maturation, developmental problems in humans, or both. On the one hand, it has been shown that it is mainly the initial Ca^{2+} rise that drives further downstream events, in particular calcium/calmodulin-dependent protein kinase II (CaMKII) action, and on the other, it is possible that this enzyme remains active even in the absence of Ca^{2+}. It therefore seems that mammalian oocytes can respond to a wide range of intracellular Ca^{2+} signals and have a surprisingly high degree of tolerance for changes in cytosolic Ca^{2+}. As epigenetic consequences or differences in gene expression have not been studied to date, artificial oocyte activation has to be considered as experimental and should only be applied with a proper indication.

KEYWORDS: artificial oocyte activation, ICSI, ionophore

It has been known for decades that periodic changes in internal Ca^{2+} mediate a large range of cell functions involved in oocyte activation and subsequent cell function. The effectiveness of these calcium oscillations depends on their number, frequency, temporal modulation and amplitude.

On the other hand, cytosolic calcium deficiency, regardless of whether the problem is sperm- or oocyte-born, will have a profound effect on cell physiology. Evidence shows that in such cases, fertilization after intracytoplasmic sperm injection (ICSI) (Ebner et al., 2012, 2015a; Montag et al., 2012) and mitotic cleavage (Ebner et al., 2015b) may be severely impaired. After all, it is generally acknowledged that intracellular Ca^{2+} is limited and, once internal stores have been depleted, the oocyte is reliant on influx of extracellular Ca^{2+} to compensate for this loss (Berridge et al., 1998). The AOA technique would facilitate such a mechanism.

As Vanden Meerschaut et al. (2014a) highlighted, AOA, in particular using Ca^{2+}ionophores such as A23187 or ionomycin, is a widely applied technique in human IVF laboratories. The problem with AOA, however, is that all application modes published to date (Vanden Meerschaut et al., 2014a) are all but standardized, which would be a prerequisite for the safe handling of oocytes in clinical use, as well as preventing meaningful comparison between different IVF studies. In general, it is interesting that artificial activation is widely used, as most of the compounds used for AOA do not result in physiological Ca^{2+} oscillations but much rather cause a single Ca^{2+} transient.

Although it is evident that oscillatory Ca^{2+} signalling is the normal stimulus during mammalian fertilization, Ducibella et al. (2003) have highlighted whether periodical changes in Ca^{2+} are indeed necessary for normal development.

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In mammalian fertilization, the spatio–temporal information about internal Ca^{2+} is transduced by downstream factors that mediate oocyte activation and subsequent preimplantation embryonic development. One such effector, CaMKII, has been found to oscillate in temporal synchrony with repetitive Ca^{2+}peaks (Markoulaki et al., 2003). This was demonstrated not only after regular fertilization but also after parthenogenetic activation (Winston and Maro, 1995) and multiple sequential ionophore pulses (Markoulaki et al., 2003). It indicates that artificial Ca^{2+} recruitment results in a proper biological downstream response. Even more interestingly, CaMKII activity obviously rises and falls with the initial Ca^{2+} transient in a directly proportional manner (Markoulaki et al., 2003), thus reflecting the situation commonly found in AOA (presence of a single calcium rise). Although oscillatory Ca^{2+} signalling may be a mechanism to maintain the sensitivity of CaMKII for the duration of Ca^{2+} signalling in regular fertilization (Ducibella et al., 2003), autophosphorylation of CaMKII, once activated, may keep the enzyme active even without the presence of calcium (Johnson et al., 1998); a scenario that would explain how a Ca^{2+} stimulus can be transmitted in the absence of its physiological repetitive pattern.

Moreover, evidence shows that, in humans, any reduction in the frequency of Ca^{2+} peaks can be compensated by both a higher amplitude and a longer total duration of the same, thus increasing the total amount of calcium released (Nikiforaki et al., 2014). This hypothesis is in agreement with mammalian data showing that oocyte activation is tolerant to perturbations in the calcium oscillation pattern as long as the total amount of calcium release is uncompromised and passes a critical threshold (Ozil et al., 2005; Toth et al., 2006). For daily practice, this would mean that an increase in length and number of exposures to ionophore could indeed show a benefit in selected cases (although this contradicts the need for a standardized procedure).

Taken together, it seems that mammalian oocytes can respond to a wide range of intracellular Ca^{2+} signalling parameters (Ducibella et al., 2003) and have a surprisingly high degree of tolerance for prolonged changes in cytosolic Ca^{2+} (Ozil et al., 2005; Toth et al., 2006).

The simple fact, however, that AOA can rescue cycles showing severe male factor infertility, deficient oocyte maturation (Kim et al., 2015), developmental problems, or both, should not mask the non-physiological nature of ionophore treatment. Given the key role of Ca^{2+} oscillations in regular ovum activation, it has been speculated that they are linked to long-term development, possibly via chromatin remodelling and reprogramming of gene expression (Ducibella et al., 2003). The observed failure in the completion of oocyte activation (Vitullo and Ozil, 1992) as well as the impairment in postimplantation viability of parthenogenetically activated mammalian oocytes (Ozil and Huneau, 2001) indicates that defective Ca^{2+} treatment could interfere with epigenetic reprogramming of the genome which, in turn, could result in an altered gene expression pattern (Ozil and Huneau, 2001). This would affect protein synthesis and degradation during the first cell cycle (Kurokawa and Fissore, 2003).

Recently, it became evident that the placenta of offspring born via assisted reproductive technologies has a reduced methylation level at H19 (Nelissen et al., 2013), which might explain the parallel finding that the epigenetic status of children conceived after IVF or ICSI differs from that after spontaneous conception (Whitelaw et al., 2014). The actual extent to which AOA, as one of the upcoming laboratory technologies, might affect DNA methylation is unclear; however, the absence of imprinting disorders in AOA live-births reported so far (Deemeh et al., 2015; Vanden Meerschaut et al., 2014b) suggests that theoretical changes in methylation might not be correlated with the overall transcriptional levels of the associated genes (Rancourt et al., 2012).

Considering the additional fact that most assisted reproduction techniques, such as ICSI, prolonged in-vitro culture or cryopreservation are thought to be related to an altered gene expression (Giritharan et al., 2010; Monzo et al., 2012), it is a realistic scenario that AOA could affect gene expression. It seems, however, to be less than expected. Compared with conventional IVF, the combination of ICSI with a chemical activation step resulted in a gene expression pattern that was found to be closer to IVF than to ICSI alone, suggesting that AOA effectively mimics, at the genetic level, a proportion of the events initiated by sperm entrance (Bridges et al., 2011).

So it is reassuring that the continuous follow-up of children born after AOA revealed that neonatal and neurodevelopmental outcome of children aged between 3 and 10 years is within expected ranges (Vanden Meerschaut et al., 2014b) and so is their language development (D'haeseleer et al., 2014).

Most likely, it is the rather inflationary use of AOA substances that has led some scientists to comment on the imminent scenario of using AOA, particularly Ca^{2+}-ionophores, as a routine procedure (Santella and Dale, 2015; van Blerkom et al., 2015). These authors consider ionophores as still "experimental", which they actually are, and the authors of this comment fully support that notion. A more open-minded commentary dealing with ionophore has stated that "it is defining modifications in treatment strategies that concurrently advances our knowledge of human fertilization and opens new avenues for research into the subcellular complexity of the earliest stages of development" (Albertini, 2015). This reflects the history of IVF, where we have to acknowledge that certain procedures were introduced without full understanding of the underlying mechanism. Moving forward does imply following a line that transfers a procedure from research to experimental to clinical. The history of AOA is probably one of the best examples and is currently in the transition process from experimental to clinical – provided that it is applied with a proper indication. To conclude, there is definitely a need for more prospective studies with a larger number of patients to confirm the observations so far and to further address the safety of AOA.

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