ASSISTED OOCYTE ACTIVATION

The technique simulating natural process of the resumption of development of fertilized oocyte.

About Assisted oocyte activation

Assisted oocyte activation (AOA) is the technique that simulates the effect of sperm penetration into an oocyte inducing oocyte activation. This technique is used in combination with intracytoplasmic sperm injection (ICSI) to improve the outcome of the fertilization process. It is mostly beneficial and recommended in those cases, when classic ICSI fails. This accounts approximately up to 5% of ICSI cycles. In most cases, the fail of ICSI is related to oocyte activation deficiency (the oocyte incapacity to continue development after fertilization). This may be due to morphologically altered sperm cells (teratozoospermia) which usually does not contain chemical substances necessary for induction of oocyte activation. This implies the use of other assisted reproduction techniques (ART) such as IMSI (morphologically selected sperm injection) prior to AOA itself. The AOA uses various methods to simulate the natural process of oocyte activation. Some assisted activation treatments such as Ca²⁺ (calcium)-ionophore, strontium chloride (vzorec) and ionomycin, promote an increase in intracellular free calcium concentrations by the release of calcium from cytoplasmic stores. Others such as electrical stimulus promote influx of calcium from the extracellular medium and some treatments such as ethanol promote both effects.

Importance of oocyte activation

Prior to fertilisation, mature oocytes remain arrested in the metaphase stage of the second meiotic division (MII). MII stage is the one corresponding with the final stage of oocyte development (oogenesis) within the ovary, the oocyte is called as mature oocyte as it is the only one with theoretical potential to undergo fertilization. Upon fusion with a sperm, MII arrest is alleviated, thereby allowing cell cycle progression, cell division, and embryogenesis to proceed. Release of meiotic arrest occurs through series of concurrent events, collectively termed oocyte activation (OA). OA converts the oocyte into a totipotent zygote, able to form all types of body cells. These morphological and biochemical events include cortical granule exocytosis (the release of granular content into the outer space) to prevent polyspermy (fertilization by more than one sperm cell), extrusion of the second polar body, maternal RNA (ribonucleic acid) recruitment (the production of oocyte’s RNA genetic information necessary for development of an embryo), pro-nuclear formation, and the initiation of embryonic development.

The main events related to oocyte activation

Apart from structural changes and meiosis resumption, there are basically two main events that characterize oocyte activation: the calcium release and the electrical events. Calcium release is the event upon which AOA focuses, as it is possible to simulate it under laboratory conditions.

The elevation of free intracellular (inside the cell) calcium is a nearly universal signal that triggers the cascade of events that leads to oocyte activation. The aim of artificial oocyte activation is to mimic physiological mechanisms, based mainly on calcium changes.

The massive increase in cytosolic calcium occurs in periodic oscillations with amplitude and frequency that are crucial for the success of oocyte activation and embryo development. Calcium oscillations cease when pronuclei are formed, which is considered to be the end point of oocyte activation.
The oocyte is electrically excitable, due to ion channels located on the plasma membrane. Changes in the electrical properties of the oocyte plasma membrane (membrane depolarization) are crucial events in the process of oocyte activation. The first calcium rise may be followed by an entry of calcium across the plasma membrane through voltage-sensitive calcium channels that respond to membrane depolarization. This entry is followed by a further depolarization, resulting from ions flowing through the plasma membrane as an ion current, which is involved in the polyspermy block (the block of all sperm cells after the penetration of the first one). The calcium oscillations are the result of the action of specific receptor located in smooth endoplasmatic reticulum. These receptors are called as IP3 Type 1 and they are activated by a signalling molecule called inositoltriphosphate (IP3). Their activation leads to calcium release. The latest advances in molecular reproduction biology show that IP3 secondary signalling molecule is gained through the action of so called phospholipase Cζ (PLCζ) which is the primary signalling molecule coming from sperm cells. This seems to be an important discovery as it seems that PLCζ may prove especially beneficial in treating specific cases of defective oocyte activation.

**Calcium ionophore treatment**

Calcium ionophore treatment is now the only commonly used method of AOA. This method of AOA focuses on direct supplementation of calcium into the oocyte using so called ionophores. These are chemical substances that reversibly bind ions. In case of AOA, calcium-binding ionophores are used, such as calcimycin or ionomycine which induce Ca2+ elevations in an oocyte. Oocyte activation with calcium ionophore may improve ICSI outcomes in infertile men.

On the other hand, using only calcium ionophore to induce oocyte activation does not mimic Ca2+ release patterns during normal fertilization as it does not stimulate the oscillatory patter of Ca2+ release. Consequently, the abnormal Ca2+ signal induced by calcium ionophores, which often manifests as a single Ca2+ transient, is a potential threat to ensuing development at later stages. Moreover, the threat is increased in cases of AOA with abnormal sperm especially in cases of sperm cells with high degree of sperm DNA fragmentation (break up of DNA into smaller functionless pieces).

**Electrostimulation**

The outcome of ICSI may be also improved by AOA by electrostimulation (electroporation). This method increases Ca2+ intracellular concentration by increasing the oocyte capacity to accept Ca2+ ions from medium surrounding the oocyte. The short, high voltage DC (direct current) pulses are known to induce a significant transmembrane Ca2+ influx (increased Ca2+ concentration within the cell) by causing a destabilization of the phospholipid bilayer (plasmatic membrane) creating pores. Electrical stimulation rescues human oocytes that failed to fertilize after ICSI and stimulates them to complete the second meiotic division, to form pronuclei (the newly formed nucleus in an early embryo development) and to undergo early embryonic development. The pattern of electrostimulation may affect embryonal development. On the other hand, there are studies reporting that higher miscarriage rate is present after AOA using electrostimulation method. To verify this method as a routine clinical procedure, further studies have to be made.

**Ethanol treatment and SrCl treatment**

Ethanol is known to induce oocyte activation. In difference of previous methods of AOA, this one combines both principals of Ca2+ influx. Ethanol induces the release of Ca2+ from oocyte’s intracellular stores by stimulating the production of IP3 and it also stimulates the Ca2+ influx from extracellular medium.

Strontium chloride (SrCl) seems to be efficient in evocating the oocyte activation even though the mechanism of its action is not yet completely elucidated. It seems that SrCl elicits Ca2+ oscillation through the synergic action of PLC activation and IP3 activity. It is documented that the use of SrCl as AOA technique may result in successful pregnancy of normozoospermic patients (patient with regular sperm production) diagnosed with OAD (oocyte activation deficiency; incapacity of an oocyte to perform activation).

**PLCζ treatment**

The phospholipase C zeta (PLCζ) was identified in the beginning of new millennium and various studies focusing on its function in oocyte activation and its potential for use in AOA have been published. As described previously, this signalling molecule (molecular messenger) is responsible of inositol triphosphate (IP3) intracellular influx necessary to induce Ca2+ oscillations typical for oocyte activation. Specifically, PLCζ is capable of transforming a chemical substance called phosphatidylinositol bisphosphate (PIP2) into IP3. In another words, PLCζ is the physiological agent responsible for IP3 mediated Ca2+ release in activating oocytes.

The PLCζ is located in the equatorial region of human sperm. Men whose sperm are unable to initiate calcium oscillations consistently fail to fertilize following ICSI and lack PLCζ. It has been established that PLCζ is the sole
Recent evidence suggests that human recombinant PLCζ may be a novel therapeutic agent for injection into the oocyte in order to rescue activation deficiency, since it promotes calcium.

**Success or failure factors**

The result of assisted oocyte activation depends on various aspects. Without any doubt, the assessment of quality of used gametes in this technique is important for assessment of its success and potential. Assisted oocyte activation is not always beneficial for patients with previous low fertilization and a suspected oocyte-related activation deficiency. For these patients, a split assisted oocyte activation-ICSI cycle using sibling oocytes can help to distinguish between a molecular oocyte-related activation deficiency and a previous technical or other biological failure. Also, there is not a general protocol available for AOA. This means that the success of its application may depend on the specifications of AOA used in each clinic.

**Complications**

At present, artificial oocyte activation is successfully applied in many IVF (in vitro fertilization) centres all over the world. Conflicting data report either risks associated with manipulating the initial stages of development, and/or reassuring healthy live births. The greatest concern arising from the use of artificial activators is potential interference with the physiological mechanisms of oocyte activation, with respect to the spatially and temporally uncontrolled action of calcium increase, its effect on cell homeostasis (physiological balance within the cell's inner ambient) and on the downstream cascade of events. These concerns, together with possible epigenetic effects that may be transmitted to the offspring, argue against routine clinical application of such manipulations for treating human infertility. Their use should instead be limited to cases of unexplained infertility or recurrent failed fertilization after ICSI. It is suggested that centres must perform artificial oocyte activation only in selected patients, such as those with PLCζ deficiency, and that the rationale for using calcium ionophore for individual cases should be documented, ensuring that patients are fully informed about the efficacy and potential risks.

**Prognosis**

In spite of the fact that gamete (generative cell; sperm cell or oocyte) activation plays a key role in fertilization, many molecular mechanisms that accompany this process remain to be elucidated. For decades, calcium release and downstream events have been recognized as playing an essential role in sperm–oocyte interaction. Artificial oocyte activation may be useful in rescuing severe male infertility and associated developmental problems. However, these advanced techniques may not be routinely available in the majority of IVF centers. At present, it is clear that the chance for successful fertilization strongly depends on fully competent gametes. Therefore, one of the major challenges in IVF lies in obtaining new accurate diagnostic tools for sperm and oocyte quality. Moving forward, assisted oocyte activation is in the same field of research as the parthenogenetic activation of the oocyte and is of great interest. The goal of parthenogenetic oocyte (the development of an oocyte without the participation of sperm cell) activation is creating human embryonic stem cell lines for use in cell and tissue therapies. Promising findings in this field have already demonstrated the potential use of stem cell lines in regenerative medicine, circumventing ethical and legal problems arising from the use of human embryonic cell lines. In any case, as assisted oocyte activation strongly depends on attributes of used gametes, identifying biomarkers of gamete quality is crucial in increasing the potential contribution of biotechnologies related to reproduction and ART technology.

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