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Failed fertilization with conventional oocyte insemination can be overcome with the ability of ICSI according to binding or failing to bind to the zona pellucida

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Summary

Purpose: To determine the frequency of failed fertilization with conventional oocyte insemination and to determine the ability of intracytoplasmic sperm injection (ICSI) to overcome the failed fertilization according to binding or failing to bind to the zona pellucida. *Materials and Methods:* Retrospective review of 12,448 in vitro fertilization (IVF) cycle to identify cycles where failed fertilization occurred following conventional oocyte insemination with seemingly normal sperm. A number of three oocytes retrieved was required. *Results:* There were only 12 cases of failed fertilization (0.1%). Six were related to failure of any or few sperm attaching to the zona pellucida These six had high fertilization rates with ICSI. Six had normal attachment and five attempted another cycle, this time with ICSI. Only 60% had good fertilization. *Conclusions:* When there is failed fertilization with normal sperm oocyte binding following conventional oocyte insemination, ICSI may still be effective in 60% of the cases, but it would be probably recommended to combine ICSI with artificial oocyte activation by calcium ionophore.

Key words: Failed fertilization; Zona pellucida; Sperm binding; Intracytoplasmic sperm injection; Calcium ionophore.

Introduction

The zona pellucida is composed of glycoproteins secreted by the oocyte. Zona protein (ZP) 3 is the most abundant. ZP3 and ZP4 are the primary ligands for sperm binding which lasts for approximately one minute. ZP2 binding occurs after the acrosome reaction and this process helps to inhibit the penetration by other sperm, i.e., prevents polyspermy [1-4].

The initial binding of the sperm to the zona pellucida requires recognition on the part of the sperm of the carbohydrate component of species specific glycoprotein ligand [4, 5]. Binding of sperm head receptors and zona pellucida ligands produce an enzyme complex which induces the acrosome reaction. The acrosome reaction releases enzymes that allow the sperm to fuse to the oocyte membrane. The fusion of sperm and oocyte membranes trigger the cortical reaction which involves the release of substances from cortical granules which are localized just below the oocyte cell membrane. The cortical reaction leads to the enzyme-induced zona reaction which causes the zona to harden. The cortical reaction also inactivates ligand for sperm receptors. This process thus inhibits penetration by more than one sperm (polyspermy).

Oocyte activation is characterized by a two-step pattern of rises in intracellular calcium (Ca^{++}) concentrations. A first Ca^{++} rise, referred to as the trigger, originates from the oocyte after sperm-oocyte membrane interaction. This initial Ca^{++} rise that is released from internal Ca^{++} stores of the oocyte membrane is dependent on a receptor-mediated interaction between the sperm and the oocyte plasma membrane [6]. With intracytoplasmic sperm injection (ICSI) this sperm oocyte plasma membrane interaction is eliminated. However, the mechanical injection procedure itself (which can occur by merely the injection without a sperm) also causes a massive influx of calcium into the oocyte and is referred to as a pseudotrigger [6].

The second step of oocyte activation that is referred to as the oscillator (related to the characteristic of a series of shorter calcium transients of high amplitude that begins 30 minutes after the trigger (step one) and continues for three to four hours) [6]. The oscillator function is dependent on the release of a sperm associated activating factor [6].

ICSI has enabled fertilization of oocytes from extremely low concentrations of viable sperm, sperm coated with a high concentration of antisperm antibodies, and immature

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testicular sperm even when taken many hours after the death of a man not on life support [7-9]. In 3% or less of cases in women who make an adequate number of follicles, there will be complete failure to fertilize the oocytes despite ICSI [10-12].

The objective of this study was to evaluate fertilization rates following ICSI in cases of failed fertilization with conventional oocyte insemination with "normal sperm" according to whether the sperm did or did not bind to the zona pellucida.

Materials and Methods

This retrospective review evaluated all in vitro fertilization (IVF) cycles where there was failed fertilization following conventional insemination with normal appearing sperm. A minimum of three oocytes retrieved was required. ICSI was offered in a succeeding IVF cycle. Fertilization rates with ICSI were then compared according to reason for failed fertilization-failure of sperm binding or failure to activate the oocyte.

Results

Twelve cases of failed fertilization were identified over a 13-year period in 12,448 IVF cycles. Six of 12 were related to very few or no sperm attached to the zona pellucida. Two cases with zona binding defects that failed to fertilize any of 16 inseminated oocytes shared a pool of oocytes with two other couples. The two male partners of these other couples fertilized 11/15 (73.3%) of the oocytes with conventional stimulation suggesting sperm receptor defect for ZP3 or ZP4 rather than mutated ZP3 or ZP4 proteins in the oocyte. ICSI negated the sperm binding defects in all six couples, showing > 50% fertilization with a total percentage of 73% (60/82). However, ICSI was not as effective with failed fertilization with normal sperm binding in two out of five couples (one did not try IVF again), showing failed fertilization (0/7) or poor fertilization (12.5%, 1/8). The other three had very good fertilization rates of 88.8% (16/18).

Discussion

Fertilization failure despite ICSI can be related to the partial or complete inability of the sperm to activate oocytes [13]. Another reason for fertilization failure despite ICSI is the inability of the oocytes to decondense the sperm [14]. Sometimes the problem is obviously related to the sperm lacking the sperm oocyte activating factor, e.g., with globozoospermia, where the fertilization rate varies from 0-37% [10, 15, 16].

Fertilization has occurred with globozoospermia following ICSI when the eggs were activated artificially by calcium ionophore [16, 17]. Cases have been described where calcium ionophore allowed activation and fertilization of an oocyte and a successful pregnancy in a couple with fertilization failure despite normozoospermic motile sperm [18, 19].

The present data showed that failed fertilization following conventional oocyte insemination with sperm with normal semen parameters is uncommon occurring in about one in 1,000 cases. Failure of sperm binding accounts for 50% of the cases and is corrected by ICSI.

ICSI injection by attaining a rapid calcium influx, overcomes phase I but not phase II oocyte activation defects. Thus when failed fertilization occurs despite normal zona binding by the sperm, a second attempt with ICSI should be accompanied by artificial activation by calcium ionophore since phase II defects may occur in about 40% of cases as suggested by these data. In fact one of the two women had marked diminished oocyte reserve. She tried Ca^{++} ionophore with ICSI in cycle 3 and fertilized the one oocyte retrieved and conceived. Unfortunately she miscarried at eight weeks despite showing fetal viability. However, she fertilized both oocytes retrieved in cycle 4 and had a normal single baby [19].

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