Artificial oocyte activation with calcium ionophore allowed fertilization and pregnancy in a couple with long-term unexplained infertility where the female partner had diminished EGG reserve and failure to fertilize oocytes despite intracytoplasmic sperm injection

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Summary

Purpose: To determine if fertilization and embryo development and pregnancy was possible following in vitro fertilization (IVF) in a couple with long-term unexplained infertility where the female partner had diminished egg reserve and where fertilization failure occurred despite conventional oocyte insemination and intracyoplasmic sperm injection (ICSI). *Methods:* In vitro fertilization was performed using a low-dose follicle stimulating hormone (FSH) stimulation protocol. Prior to ICSI, artificial oocyte activation with calcium ionophore was used. *Results:* Only one mature oocyte was retrieved but it fertilized and cleaved to a good quality 8-cell embryo on day 3. A pregnancy with fetal viability was achieved but she subsequently miscarried. A second attempt successful. *Conclusions:* Fertilization and pregnancy is possible even in women with diminished egg reserve with previous failed fertilization with ICSI by performing artificial oocyte activation with calcium ionophore. It is not clear if the sperm lacked oscillin or if the eggs were not responsive to oscillin.

Key words: Artificial oocyte activation; Calcium ionophore; Normal sperm; Diminished egg reserve; Minimal stimulation.

Introduction

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 [1].

With intracytoplasmic sperm injection (ICSI) the sperm oocyte plasma membrane interaction is eliminated. However, the mechanical injection procedure itself (which can occur by merely the injection without sperm) also causes a massive influx of calcium into the oocyte and is referred to as a pseudotrigger [1].

The second step of oocyte activation 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 [1]. The oscillator function is dependent on the release of a sperm associated activating factor. This factor has been named oscillogen or oscillin [2]. This sperm oocyte activating factor can control the frequency of oscillations (thus the name oscillin). It is not species-specific and thus a deficiency of oscillin in human sperm can be detected by failure to activate mouse oocytes [3]. This test is referred to as the mouse oocyte activation test or MOAT. Sperm demembranization is necessary to allow the release of the cytosolic sperm factor responsible for the oscillator function and its sustained activity [4, 5].

ICSI has enabled fertilization of oocytes from extremely low concentrations of viable sperm, sperm coated with a high concentration of antisperm antibodies, and immature testicular sperm even when taken many hours after the death of a man not on life support [6-8]. 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 [9-11].

Fertilization failure despite ICSI can be related to the partial or complete inability of the sperm to activate oocytes [12]. Another reason for fertilization failure despite ICSI is the inability of the oocytes to decondense the sperm [13]. Sometimes the problem is obviously related to the sperm lacking oscillin, e.g., with globozoospermia where the fertilization rate varies from 0-37% [9, 14, 15]. Fertilization has occurred with globozoospermia following ICSI when the eggs were activated artificially by calcium ionophore [15, 16]. A case was 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 [17].

The case described here reports another situation where there was complete fertilization failure despite normal sperm and ICSI where oocyte activation with calcium ionophore was performed followed by fertilization with ICSI leading to a pregnancy only this time in a woman with diminished egg reserve.

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Case Report

A 34-year-old female married for 14 years and having unprotected sex for 12 years presented with primary infertility. She lived in another state and was found to be "approaching menopause" with a serum FSH of 64 mIU/ml. A second serum FSH was 37 mIU/ml with a serum estradiol (E2) of 56 pg/ml. She still had spontaneous menses but had oligomenorrhea. The only interruption to her attempts at fertility was a one and a half respite while she was evaluated and treated for an ocular pseudotumor.

The couple had insurance coverage for in vitro fertilization (IVF) but not for donor egg coverage. The reproductive center that they had consulted presented data from one of the leading IVF centers in the world claiming no live pregnancies despite the transfer of normal appearing embryos in women of any age if the serum FSH was > 15 mIU/ml [18]. They refused to try any treatment other than the use of donor oocytes which the couple could not afford.

The couple was aware of the data from our IVF center that found quite reasonable pregnancy rates with IVF-ET in women whose mean day 3 serum FSH was much higher than 15 mIU/ml as long as a lower dosage of FSH was used for follicular maturation [19]. Though there did not appear to be any problem in the semen analysis or fallopian tubes, because of the length of their infertility and their insurance coverage for IVF-ET we recommended IVF-ET.

The initial semen analysis at our facility showed a normal semen concentration of 32.7 million per ml with 70.6% motility with 18% with rapid linear motion. The hypo-osmotic swelling test was normal at 68% and there were no antisperm antibodies. The one "abnormality" was that the percentage of sperm with normal morphology using strict criteria was only 4% [20]. The couple was advised that for many years some clinicians believed (and some still believe this concept today) that when the sperm morphology was ≤ 4% that neither intercourse, intrauterine insemination or conventional oocyte insemination will result in adequate pregnancy rates [20]. In fact they had concluded the pregnancy rates approached zero [21-24]. However, they were also advised that our own data failed to corroborate these previous studies and in fact found no predictive value of strict morphology ≤ 4% [25-27]. In fact in another study we actually found that there was no greater risk of failed fertilization with conventional oocyte insemination with teratozoospermia sperm compared to ICSI but the former resulted in significantly higher pregnancy rates per transfer [11].

For cycle 1 a protocol was used that used a little more FSH than the usual minimal stimulation used for diminished egg reserve based on a more optimistic baseline blood with a serum E2 of 87.9 pg/ml and a serum FSH of 11 mIU/ml [28]. The peak serum E2 reached 331 pg/ml and two mature oocytes were inseminated by conventional insemination. The couple chose this fertilization option because of the data showing higher pregnancy rates with conventional oocyte insemination and to determine if their long-term infertility failure could be related to failed fertilization [11]. Indeed these oocytes failed to fertilize.

For cycle 2 a lower dose FSH protocol was used and was started on day 5 when the serum E2 was 54.2 pg/ml and the serum FSH was 14.3 mIU/ml. For both cycles the serum FSH was lowered with ethinyl estradiol [30, 31]. Three mature follicles were retrieved but none fertilized despite ICSI. Semen parameters were completely normal.

For her third and final insurance covered cycle we suggested artificial oocyte activation with calcium ionophore. For cycle 3 her day 2 serum E2 was 38.6 and the serum FSH was 12.6 mIU/ml while taking ethinyl estradiol (20 μg) daily. With a minimum stimulation protocol her peak serum E2 reached 200 pg/ml. A single metaphase II egg was retrieved. Artificial oocyte activation was performed using calcium ionophore as previously described [15-17]. An 8-cell embryo on day 3 with 25% fragmentation was transferred and she conceived. She showed fetal viability six weeks from egg retrieval with properly rising serum beta human chorionic gonadotropin levels. However, unfortunately she subsequently had a miscarriage. Knowing now what is needed to achieve a pregnancy the couple tried again using a low-dose FSH protocol because of diminished egg reserve and artificial oocyte activation with calcium ionophore. Two oocytes fertilized and two embryos (a 5-cell and 8-cell) were transferred and she has completed the first trimester.

Discussion

It is clear that women with markedly elevated FSH levels, even over 100 mIU/ml, and even those in apparent menopause, can achieve successful pregnancies [29-35]. Those who believe that an elevated serum FSH results in embryos that do not implant still recognize that there is generally no problem with fertilization [18]. Though it is clear that the probable cause of the couple's long-term infertility was the inability of the sperm to fertilize the egg, it is not clear whether the problem was a sperm or oocyte factor.

The problem of failed fertilization should not have been related to the first step of fertilization, i.e., the trigger rise of Ca^{++} that occurs with contact of the sperm with the oocyte membrane since the first cycle using conventional insemination demonstrated no problem with sperm binding. Furthermore, even if for some reason binding of sperm did not cause the trigger, then the defect should have been obviated by the mechanical injection of the oocyte membrane by ICSI (pseudotrigger) in cycle 2.

Most of the data suggests that Ca^{++} ionophore treatment of oocytes has a reasonable chance of allowing fertilization and normal embryo development and even pregnancies when the sperm are deficient in oscillin. However, there are some data suggesting that Ca^{++} ionophore treatment can also allow fertilization when the problem with failed fertilization is related to oocyte rather than sperm defects [36].

If calcium ionophore proves less efficient for oocyte vs sperm-related defects leading to failed fertilization there is another technique that has been shown to enable fertilization when the egg does not respond to proper sperm signals [37]. Fertilization and embryo development and pregnancies have been reported using a modified ICSI technique using vigorous aspiration of oocyte cytoplasm when failed fertilization has been related to a defective oocyte factor that is involved in the activation of the oscillator mechanism [37]. Comparative studies in a larger series which would require a cooperative study between many IVF centers could help to determine which technique is more efficacious for sperm or oocyte related defects in the oscillator mechanism of fertilization and whether one technique is safer than the other. When calcium ionophore is used the patient should be advised that there is limited information of its potential toxic effect on oocytes and embryos.

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