Original Articles

Reproductive Biology Section

Correction of failed fertilization despite intracytoplasmic sperm injection with oligoasthenoteratozoospermia but with acrosomes present by oocyte activation with calcium ionophore - case report

J. Pinto, *B.A.*; **J.H. Check,** *M.D., Ph.D.*

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: To determine if fertilization and embryo cleavage can be achieved by artificial oocyte activation in circumstances of repeated failed fertilization with sperm that have an acrosome. *Methods:* A woman with three IVF cycles with intracytoplasmic sperm injection (ICSI) failed to fertilize any eggs. The sperm had severe oligoasthenoteratozoospermia with no sperm with normal morphology. In the fourth IVF cycle fertilization was evaluated by performing ICSI with the husband's sperm and egg activation with calcium ionophore, ICSI with the husband's sperm without artificial oocyte activation, and ICSI with donor sperm. *Results:* Five mature oocytes were retrieved. Of the four eggs having ICSI with the husband's sperm only one of the two activated by calcium ionophore fertilized and resulted in a cleaved day 3 embryo. Interestingly, the one egg fertilized by donor sperm did not fertilize. *Conclusions:* The data could be consistent with conclusions that in some cases the failure to fertilize may be related to an oocyte activation factor/receptor problem in the oocyte that can be overcome by the use of calcium ionophore.

Key words: Artificial oocyte activation; Failed fertilization; Calcium ionophore; Acrosomes.

Introduction

Successful fertilization depends on sperm and oocyte interactions: sperm-zona binding, zona-induced acrosome reaction, sperm-zona penetration and sperm-oolemma binding [1]. Of these interactions, the acrosome reaction has been shown to be a requirement for normal fertilization. Sperm that lack acrosomal vesicles and are unable to bind to or penetrate the zona pellucida, commonly result in fertilization failure following in vitro fertilization (IVF) [1]. The physiological stimuli for the acrosome reaction involve the zona pellucida protein 3 (ZP3) [2]. Thus, when sperm are unable to reach the zona pellucida, either an external activating factor or another pathway towards activation must be utilized.

In the past, men who had this type of male factor defect were considered irreversibly infertile. The advent of intracytoplasmic sperm injection (ICSI) circumvented capitation and the acrosome reaction. Intracytoplasmic sperm injection involves the direct injection of spermatozoa into the oocyte, increasing fertilization rates to approximately 70% as reported by one study [3]. However, there is still a 2-3% failure rate with ICSI [4]. Such a failure rate has been explained by a lack of oocyte activation [5].

In normal conception, oocyte activation occurs by a two-signal process. The acrosome reaction stimulates the initial signal of Ca^{2+} release. In ICSI, injecting the spermatozoa into the oocyte stimulates signal 1. The interaction between fertilizing spermatozoa and oocyte then triggers the release of Ca^{2+} (signal 2) in an oscillatory fashion [6-8]. It is not known how the sperm activates the fertilization calcium wave. Two theories have arisen: sperm either activates a signal transduction receptor on the oocyte surface or transports an active messenger, in the form of a soluble sperm factor (SSF) [8, 9]. In failed ICSI cycles, the lack of adequate SSF may be overcome by using a calcium ionophore booster [8-11]. Calcium ionophore serves as nonphysiologic stimuli of the acrosome reaction, thus affecting signal 1 of oocyte activation. Furthermore, it increases the oocyte calcium load, allowing for the development of Ca^{2+} oscillations (signal 2) [11]. Activation of human oocytes using calcium ionophore improves fertilization rates of cases with poor ICSI fertilization [8-11]. In failed ICSI cycles with calcium ionophore, it may be inferred that while there are adequate stores of calcium, the oocyte calcium receptor is defective.

In this case report, we present a couple with prolonged unexplained infertility with repeated IVF and ICSI fertilization failure and compare the effect of using calcium ionophore on fertilization and cleavage on oocytes insem-

Revised manuscript accepted for publication September 20, 2007

inated by ICSI versus two controls: donor sperm with ICSI and the husband's sperm with ICSI without calcium ionophore.

Case Report

A gravida 0, para 0, 32-year-old woman and her 33-year-old husband were referred to our IVF center after three failed IVF cycles with ICSI at another center. A total of 17 eggs with seven mature eggs were produced in the three cycles. No viable embryos were produced from the three cycles and thus no embryo transfers. One oocyte had two pronuclei but failed to cleave. The couple had been married for five years with four years of primary infertility. The wife's physical and gynecological examinations were within normal limits, including sonohystogram, hysterosalpingogram, ovarian egg reserve tests, and thyroid function tests. Her menses occurred at regular intervals approximately 27 to 29 days apart. The semen analysis showed a volume of 1.1 ml; count of 0.4 mill/ml; motility of 0%; and sperm viability of 75%. Morphology studies showed that the sperm were 100% abnormal but were not round-headed sperm in that they retained an acrosome. The husband underwent further hormonal testing which all came back as normal with the following values – FSH of 7.3 mIU/ml; LH of 4.6 mIU/ml; estradiol of < 30 pg/ml; testosterone of 348 ng/dl and free testosterone of 67.7 pg/ml. Given that previous cycles of IVF had failed and the low numbers in the semen analysis, we suggested the use of ICSI with calcium ionophore and/or donor sperm with ICSI, following minimal stimulation.

One consideration for the previous failures was that the controlled ovarian hyperstimulation regimen may have adversely affected the fertilization process. Previous data did find that the longer use of leuprolide acetate could inhibit conventional fertilization of eggs [12]. Though there is evidence that the use of gonadotropins can be associated with diminished fertility it is usually considered to be related to an adverse effect on the uterine environment especially in women with normal egg reserves [13, 14]. The mid-luteal phase leuprolide acetate protocol was used in the three previous failed IVF with ICSI cycles in the other center. Thus she was placed on the minimal stimulation protocol starting with 75 IU of FSH on day 3 and used a total of 975 IU of gonadotropins. The gonadotropin releasing hormone antagonist cetrorelix (250 mcg/day) was initiated when the follicle reached 12 mm on day 7 of her cycle. Five oocytes were retrieved. All oocytes were used. Four oocytes were injected with her husband's sperm through ICSI. Two of the four oocytes received calcium ionophore. The fifth oocyte was injected with donor sperm with ICSI and without calcium ionophore. The only viable embryo produced resulted from injection of the oocyte with her husband's sperm and supplementation with calcium ionophore. The embryo was then transferred on day 3 at the 6-cell stage but failed to achieve a pregnancy.

Discussion

The failure to fertilize any eggs that appeared morphologically normal after three IVF with ICSI attempts using sperm with severe oligoasthenozoospermia would normally lead to the conclusion that a sperm defect was responsible for the failed fertilization. Even though ICSI has resulted in successful fertilization and pregnancies with extremely poor sperm, e.g., non-motile stick-like sperm, obtained from testicular aspiration posthumously from a man who had gone almost 24 hours without life support, the ICSI process may not be a panacea for all male factor problems [15].

Some round headed sperm without apparent acrosomes have been able to fertilize eggs with ICSI though some can only do so with artificial egg activation, e.g., with calcium ionophore [16-20] and some completely fail [21]. This difference may be related to whether there is complete absence of the acrosome or at least a small portion of a critical area of the acrosome still present. Theoretically, however, sperm with perfectly normal appearance of the acrosomes could fail to fertilize even with ICSI implying a defect in the oocyte activating factor (OAF) in the sperm or a receptor defect in the oocytes.

The present case could still be interpreted as a male with severe oligoasthenoteratozoospermia who lacks the OAF and is thus helped by the use of calcium ionophore. The failure to fertilize the egg by donor sperm could be explained possibly by the fortuitous selection of one poor egg or some inefficiency in the mechanism of the ICSI process on that one egg. One hundred percent fertilization by ICSI even with perfectly normal sperm is not common.

However, these data could also be interpreted as to the possibility that there could be some women with defective oocytes that are insensitive to sperm triggering signal 2 of calcium release. However, fertilization is possible with a more potent stimulus, e.g., calcium ionophore.

The continued failure to fertilize any eggs with the male partner's sperm or donor sperm is sufficiently convincing that the previous three failed IVF-ICSI cycles were not related to the controlled ovarian hyperstimulation (COH) regimen and that the successful fertilization of the two oocytes was related to the addition of the calcium ionophore rather than the use of a minimal stimulation regimen. The plan is for her next IVF cycle to use traditional COH but to use a gonadotropin-releasing hormone antagonist rather than an agonist to make eggs available for ICSI. The plan is to attempt to fertilize 75% of the eggs with the male partner's sperm with ICSI and artificial egg activation with calcium ionophore and 25% with donor sperm and ICSI but no artificial egg activation. If once again the male partner's sperm with the help of calcium ionophore fertilizes the eggs but donor sperm again fail to fertilize then it can be concluded that in some cases an oocyte defect exists where there is relative insensitivity to the normal sperm in the oscillation phase of calcium release. If on the other hand the donor sperm does fertilize, then this would evidence that some sperm with an acrosome present may still lack the ability to activate the oocyte. However, fertilization under these circumstances with cleavage to normal appearing embryos is still possible using artificial egg activation.

References

[1] Baker H.W.G., Liu D.Y., Garrett C., Martic M.: "The human acrosome reaction". *Asian J. Androl. 2000,* 2000, *2*, 172.

[2] Allgeyer M., Kirschenhofer N., Mann U., Brucker C.: "Measurement of induced acrosome reactions in human sperm using physiologic stimuli - relevance for the prediction of fertilization outcome". *Andrologia,* 2006, *38,* 99.
[3] Ben-Yosef D., Shalgi R.: "Oocyte activation: lessons from human infertility". *Trends Mol. Med.,* 2001, *7,* 163.
[4] Mahutte N.G., Arici A.: "Failed fertilization: is it predictable?". *Curr. Opin. Obstet. Gynecol.,* 2003, *15,* 211.
[5] Murase Y., Araki Y., Mizuno S. *et al*.: "Pregnancy following chemical activation of oocytes in a couple with repeated failure of fertilization using ICSI: Case report". *Hum Reprod.,* 2004, *19,* 1604.
[6] Lewis S., Klonoff-Cohen H.: "What factors affect intracytoplasmic sperm injection outcomes?". *Obstet Gynecol Survey*, 2005, *60*, 111.
[7] Tesarik J. Mendoza C.: "In vitro fertilization by intracytoplasmic sperm injection". *BioEssays.,* 1992, *21*, 791.
[8] Sousa M., Mendoza C., Barros A., Tesarik J.: "Calcium responses of human oocytes after intracytoplasmic injection of leukocytes, spermatocytes, and round spermatids". *Mol. Hum. Reprod*., 1996, *11,* 853.
[9] Whitaker M.: "Calcium at fertilization and in early development". *Physiol Rev.,* 2006, *86*, 25.
[10] Heindryckx B., Van der Elst J., Sutter P.De., Dhont M.: "Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI". *Hum. Reprod.,* 2005, *8,* 2237.
[11] Tesarik J., Testart J.: "Treatment of sperm-injected human oocytes with Ca^{2+} ionophore supports the development of Ca^{2+} oscillations". *Biol. Reprod.,* 1994, *51,* 385.
[12] Check J.H., Long R., Allen J., Hoover L.: "Poor fertilization may be related to oocyte or zona pellucida recognition defects specific to certain hyperstimulation regimens and limited to some males but not to others: a case report". *Fertil. Steril.,* 1995, *63*, 1341.
[13] Check J.H.: "Progesterone therapy versus follicle maturing drugs – possible opposite effects on embryo implantation". *Clin. Exp. Obstet. Gynecol.,* 2002, *29*, 5.
[14] Check J.H., Choe J.K., Katsoff D., Summers-Chase D., Wilson C.: "Controlled ovarian hyperstimulation adversely affects implantation following in vitro fertilization-embryo transfer". *J. Assist. Reprod. Genet.*, 1999, *16,* 416.
[15] Check M.L., Check J.H., Summers-Chase D., Choe J.K., Check D.J., Nazari A.: "Live birth after posthumous testicular sperm aspiration and intracytoplasmic sperm injection with cryopreserved sperm: Case report". *Clin. Exp. Obstet. Gynecol.*, 2002, *29,* 95.
[16] Liu J., Nagy Z., Joris H., Tournaye H., Devroey P., Van Steirteghem A.C.: "Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globoospermia". *Hum. Reprod.*, 1995, *10,* 626.
[17] Stone S., O'Mahony F., Khalaf Y., Taylor A., Braude P.: "A normal live birth after intracytoplasmic sperm injection for globozoospermia without assisted oocyte activation: case report". *Hum. Reprod.,* 2000, *15*, 139.
[18] Kilani Z.M., Shaban M.A., Ghunaim S.D., Keilani S.S., Dakkak A.I.: "Triplet pregnancy and delivery after intracytoplasmic injection of round-headed spermatozoa". *Hum. Reprod.,* 1998, *13*, 2177.
[19] Battaglia D.E., Koehler J.K., Klein N.A., Tucker M.J.: "Failure of oocyte activation after intracytoplasmic sperm injection using round-headed sperm". *Fertil. Steril.,* 1997, *68,* 118.
[20] Rybouchkin A.V., Van der Straeten F., Quatacker J., De Sutter P., Dhont M.: "Fertilization and pregnancy after assisted oocyte activation and intracytoplasmic sperm injection in a case of round-headed sperm associated with deficient oocyte activation capacity". *Fertil. Steril.*, 1997, *68,* 1144.
[21] Check J.H., Levito M.C, Summers-Chaser, D., Marmar J., Barci H.: "A comparison of the efficacy of intracytoplasmic sperm injection (ICSI) using ejaculated sperm selected by high magnification versus ICSI with testicular sperm both followed by oocyte activation with calcium ionophore: case report". *Clin. Exp. Obstet. Gynecol.,* 2007, *34,* 111.

Address reprint requests to:
J.H. CHECK, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)
e-mail: laurie@ccivf.com