Original Articles

Reproductive Biology Section

Pregnancy following calcium ionophore oocyte activation in an oligozoospermia patient with repeated failure of fertilization after ICSI

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Summary

A successful pregnancy outcome after calcium ionophore A23187 oocyte activation in an infertile couple with a repeated failure of achieving fertilization after ICSI is reported. The secondary infertility couple with oligozoospermia underwent ICSI two times. However, none of the oocytes were fertilized. In the third ICSI attempt, three oocytes after ICSI were activated using calcium ionophore for five minutes. Two of three oocytes thus became fertilized. A successful pregnancy outcome was thereafter achieved with the delivery of a healthy infant without congenital abnormalities. Oocyte activation using calcium ionophore was thus found to be a useful method in a case of repeated failure of fertilization after ICSI.

Key words: Repeated fertilization failure; ICSI; Oocyte activation; Calcium ionophore.

Introduction

Intracytoplasmic sperm injection (ICSI) is an extremely useful method of treating patients who have an extremely low sperm count, and it has been reported that the fertilization rate after ICSI is 64% to 71% [1, 2]. Conversely, it has been reported that 3% of all cycles are observed to result in no fertilized oocyte, despite the performance of ICSI [3, 4].

One cause of fertilization failure after ICSI is believed to be oocyte activation failure attributable to the sperm. Correspondingly, it has recently been reported that fertilization can be achieved by using calcium ionophore to activate the oocyte, thereby obtaining favorable results [5-8]. A case of secondary infertility is reported in which fertilization had not been achieved despite the performance of ICSI but wherein fertilization was achieved by using calcium ionophore to activate the oocyte after ICSI.

Case Report

A 40-year-old female and her 47-year-old husband presented at the Niigata Workmen's Accident Hospital for secondary infertility of two years duration. The patient had previously conceived in intrauterine insemination and delivered a normal mature infant. Her menstrual cycles were regular and her hormonal testing was normal. The semen analyses revealed oligozoospermia (motile sperm: 0.5-1.5 x 10^6/ml). Thereafter the patient elected to undergo ICSI.

The patient underwent ovarian stimulation with 100 mg clomiphene citrate on days 4-8 of her menstrual cycle and 150 IU of HMG (HMG Nikken; Nikken Chemicals Co., LTD., Japan) injection on day 8 and day 10. Two mature oocytes were retrieved. Motile sperm with no obvious abnormal morphology were injected into two metaphase II (MII) oocytes using the routine ICSI procedure. However, none of the oocytes became fertilized.

In the second attempt, the patient underwent ovarian stimulation with 100 mg clomiphene citrate on days 4-8 and 150 IU of HMG injection on day 8, day 10 and day 12 of her menstrual cycle. As a result three oocytes were retrieved. Motile sperm were injected into two MII oocytes. However, none of the oocytes became fertilized.

In the third attempt, the patient underwent ovarian stimulation with 100 mg clomiphene citrate on days 4-8 and 150 IU of HMG injection on day 8 and day 10 of her menstrual cycle. As a result, three mature oocytes were retrieved. We performed calcium ionophore oocyte activation after obtaining informed consent. Motile sperm were injected into three MII oocytes. After 30 min of the ICSI procedure, three oocytes were exposed to 5 µmol/l of calcium ionophore A23187 (Sigma, St. Louis, MO) in HFF99 (Fuso Pharmaceutical Industries, Ltd., Japan) medium with 10% SSS (Irvine, Santa Ana, CA) for 5 min at 37°C in 5% CO_2/5% O_2. The oocytes were then washed in fresh media and incubated overnight at 37°C in 5% CO_2/5% O_2 in HFF99 medium with 10% SSS. This time, two of three oocytes became normally fertilized (two pronuclei). One oocyte had three pronuclei. One embryo transfer was performed on day 3 of culture. The patient thereafter conceived and delivered a 2,520 g healthy baby without any congenital abnormalities at 36 weeks of gestation.

Discussion

For a case of secondary infertility in which fertilization could not be achieved despite the performance of ICSI, calcium ionophore was used to activate the oocyte after ICSI. Thereby, fertilization was achieved, thus leading to pregnancy and a successful delivery. Recently, it has been reported that in cases in which fertilization could not be achieved via ICSI, oocyte activation has been performed

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using calcium ionophore, and subsequent fertilization has been achieved leading to pregnancy and successful delivery [5-8]. In round-head spermatozoa (globozoospermia) with an abnormal shaped sperm head, fertilization rate after ICSI is low, and there has been the report of a case in which oocyte activation was performed using calcium ionophore [8]. There has also been the report of a case in which fertilization was not achieved via the usual ICSI despite a normal motility rate and shape of the sperm, but oocyte activation using calcium ionophore was performed, thus leading to pregnancy [6]. It is believed that this case is one in which the condition of sperm previously having a fertilizing capacity changes over time thereafter, thus resulting in the loss of oocyte activation capacity. To the extent of our search of the pertinent medical literature, there have not been any reports of cases in which oocyte activation has been performed for such cases, and thereby resulting in a live birth, so the present case constitutes the first such report.

When considering whether the cause of oocyte activation failure after ICSI is attributable to the sperm, it is necessary to examine mainly whether there is any abnormality in the oocyte activation factor within the sperm. Recently, as for the oocyte activation factor within the sperm, phospholipase Cζ (PLCζ) has been reported as a potential candidate, which has been attracting attention [9]. This refers to the fact that fertilization is achieved by oocyte activation due to PLCζ which is brought into the oocyte cytoplasm after ICSI.

In the present case, since the first fetus was conceived via intrauterine insemination and a live birth was achieved, it is believed the sperm had a normal fertilizing capacity. Thereafter, the sperm count decreased, leading to an indication for ICSI, after which it was determined to administer the treatment. The first and second ICSI both constituted ICSI using normal shaped sperm having satisfactory mobility, but no fertilization was observed. It is commonly believed that the cause of fertilization failure may be an abnormality in the quality of the oocyte or an oocyte activation failure attributable to the sperm, but the oocyte that was used in the first and second ICSI had a morphologically favorable condition. Therefore, oocyte activation failure attributable to the sperm might have been the cause of the fertilization failure. One method for proving oocyte activation failure attributable to the sperm is to verify the presence or absence of oocyte activation by performing ICSI for the provided oocyte, but this was not ethically possible. Even though it was not clinically possible to prove oocyte activation failure attributable to the sperm, it was determined that artificial oocyte activation was necessary, and a course of using calcium ionophore to activate the oocyte was taken. After performing ICSI for three mature oocytes and then using calcium ionophore to activate the oocytes, normal fertilization was thus observed in two oocytes. Pregnancy was achieved via implantation, thereafter leading to a successful live birth.

Conclusion

In this study, calcium ionophore was used to activate the oocytes for a case demonstrating secondary infertility of fertilization failure after ICSI and thus a live birth was achieved. The present method is therefore believed to be extremely useful for cases of fertilization failure after ICSI.

References

[1] Tarlatzis B.C., Bili H.: "Intracytoplasmic sperm injection. Survey of world results". *Ann. NY. Acad. Sci*., 2000, *900,* 336.
[2] Van Steirteghem A., Nagy P., Joris H., Janssenswillen C., Staessen C., Verheyen G., Camus M., Tournaye H., Devroey P.: "Results of intracytoplasmic sperm injection with ejaculated, fresh and frozen-thawed epididymal and testicular spermatozoa". *Hum. Reprod*., 1998, *13* (suppl. 1), 134.
[3] Liu J., Nagy Z., Joris H., Tournaye H., Devroey P., Van Steirteghem A.: "Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia". *Hum. Reprod*., 1995, *10,* 626.
[4] Flaherty S.P., Payne D., Matthews C.D.: "Fertilization failures and abnormal fertilization after intracytoplasmic sperm injection". *Hum. Reprod*., 1998, *13* (suppl. 1), 155.
[5] Nakagawa K., Yamano S., Moride N., Yamashita M., Yoshizawa M., Aono T.: "Effect of activation with Ca ionophore A23187 and puromycin on the development of human oocytes that failed to fertilize after intracytoplasmic sperm injection". *Fertil. Steril*., 2001, *76,* 148.
[6] Eldar-Geva T., Brooks B., Margalioth E.J., Zylber-Haran E., Gal M., Silber S.J.: "Successful pregnancy and delivery after calcium ionophore oocyte activation in a normozoospermic patient with previous repeated failed fertilization after intracytoplasmic sperm injection". *Fertil. Steril*., 2003, *79* (suppl. 3), 1656.
[7] Murase Y., Araki Y., Mizuno S., Kawaguchi C., Naito M., Yoshizawa M., Araki Y.: "Pregnancy following chemical activation of oocytes in a couple with repeated failure of fertilization using ICSI: case report". *Hum. Reprod*., 2004, *19*, 1604.
[8] Kim S.T., Cha Y.B., Park J.M., Gye M.C.: "Successful pregnancy and delivery from frozen-thawed embryos after intracytoplasmic sperm injection using round-headed spermatozoa and assisted oocyte activation in a globozoospermic patient with mosaic Down syndrome". *Fertil. Steril*., 2001, *75*, 445.
[9] Saunders C.M., Larman M.G., Parrington J., Cox L.J., Royse J., Blayney L.M., Swann K., Lai F.A.: "PLC zeta: a sperm-specific trigger of Ca(2+) oscillations in eggs and embryo development". *Development*, 2002, *129*, 3533.

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