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ASSISTED REPRODUCTION TECHNOLOGIES

Live birth after $SrCl_2$ oocyte activation in previous repeated failed or low fertilization rates after ICSI of frozen-thawed testicular spermatozoa: case report

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Abstract
Purpose To report a live birth resulting after strontium chloride ($SrCl_2$) oocyte activation in a couple with complete fertilization failure or low fertilization rates following intracytoplasmic sperm injection (ICSI) of frozen-thawed testicular spermatozoa.
Methods The couple underwent ICSI of frozen-thawed testicular spermatozoa. After ICSI, the oocytes were artificially activated by $SrCl_2$ because the results of fertilization were not satisfactory in the previous cycles. The main outcome measures were fertilization, pregnancy, and birth.
Results In the first and second cycles performed previously at another clinic, fertilization rates were 9.1 % and 0.0 %, respectively. In the third cycle, 31 metaphase II oocytes were retrieved. After sperm injection, all of the oocytes were stimulated using $SrCl_2$ for activation. Sixteen oocytes were fertilized (51.6 %), and a single embryo was transferred into the uterus on Day 3. A healthy girl weighing 2750 g was born at 40 weeks of gestation by caesarean section.
Conclusions This result suggests that $SrCl_2$ could be useful for oocyte fertilization in case of repeated complete fertilization failure or low fertilization rates following ICSI of frozen-thawed testicular spermatozoa.

Capsule Artificial oocyte activation with $SrCl_2$ improves fertilization, pregnancy and birth in a couple with low fertilization rates following ICSI of frozen-thawed testicular spermatozoa.

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Keywords Artificial oocyte activation (AOA) · Fertilization failure · Frozen-thawed testicular spermatozoa · ICSI · $SrCl_2$

Introduction

Intracytoplasmic sperm injection (ICSI) has become the most effective therapeutic treatment for severe male-factor infertility. The fertilization rate of ICSI is typically considered to be the highest among the assisted reproduction techniques presently being performed. Therefore, azoospermia can be treated successfully with fresh or frozen testicular sperm extraction (TESE)-ICSI if enough amount of spermatozoa were obtained from testicular biopsy. However, we occasionally encountered the unusual case in which fertilization failed despite the sperm being properly injected into the oocyte, and it has been a difficult problem to solve for a long time. The reasons for this phenomenon was considered to be a partial or complete inability of the spermatozoa to activate the oocytes, deficiency of sperm protamine, or the inability of the oocytes to decondense spermatozoa [1–3]. When the oocytes were activated using electroporation [4, 5], calcium ionophore [6–14], or calcium ionophore and puromycin [15, 16], followed by ICSI in women whose oocytes failed to fertilize in previous IVF cycles, some of them could form pronuclei. Recently, several studies were reported that strontium chloride ($SrCl_2$) treatment on infertile patients resulted in successful pregnancies and deliveries [17–19]. Especially, $SrCl_2$ is excellent for improving fertilization, embryo quality, and pregnancy in women who showed complete fertilization failure or low fertilization rates. Furthermore, the physical and mental development of these children from birth to

12 months were normal [18]. We report a case of successful fertilization after artificial oocyte activation (AOA) by $SrCl_2$ in ICSI of frozen-thawed testicular spermatozoa.

Case report

This study was performed with the approval of the Institutional Review Board of Maria Fertility Hospital and with the informed consent of the couple. A 32-year-old woman and her 37-year-old husband with a 2-year history of infertility were referred to our IVF center for treatment. The woman's physical and gynecological examinations were within normal limits, including hysterosalpingography and routine blood tests. Her husband displayed a unilateral testicular appendix (not cancer), and semen analysis revealed azoospermia. It was supposed to be due to inflammation or others reason, and had been removed. Testicular biopsy was performed before the initiation of the cycle to ensure having sperms on hand. He underwent TESE by 5-gauge needle aspiration, and a total of 0.01×10^6 spermatozoa were mixed with sperm freezing medium (Irvine Scientific, Santa Ana, CA) and were frozen with a computerized freezer (CryoMaigic, Mirae Biotech, Korea) for 30 min. The frozen sperms were thawed by removing the cryogenic vials from liquid nitrogen and by immersing in 37 °C water bath.

Because the first and second cycles had been performed at another clinic, there were no available data in detail. In the previous cycles, fertilization rates were 9.1 % (2/22) and 0.0 % (0/17), respectively, and thus, pregnancy was not achieved.

In the third cycle, ovarian stimulation was conducted using a combination of gonadotrophin-releasing hormone (GnRH) agonist (leuprorelin; Lucrin Depot, Abbott Laboratories, Spain) and human menopausal gonadotrophin (hMG) (Merional, Institute Biochemique SA, Switzerland). An injection of 10,000 units of human chorionic gonadotrophin (hCG) (IVF-C, LG Chem, Korea) was administered when the dominant follicle reached a mean diameter of 18 mm. Oocyte retrieval was performed 36 h after hCG administration. Transvaginal ultrasound-guided aspiration was performed with a 19-gauge needle. A total of 32 oocytes were retrieved. Thirty-one of these oocytes were in metaphase II oocytes, and ICSI was performed using the frozen-thawed testicular sperm. There were very few motile sperms. Most of the immotile sperms were structurally abnormal and had no flexible tail. All injected oocytes were stimulated using 10 mM of $SrCl_2$ (Sigma-Aldrich, St Louis, MO, USA) for 60 min, approximately 30 min after oocyte activation by ICSI. Oocytes were subsequently rinsed several times in Sydney IVF fertilization medium (Cook, Brisbabe, Australia). Fertilization was assessed 18 h after insemination by the appearance of two distinct pronuclei and two polar bodies. The zygotes were cultured with 10 μl of Sydney IVF cleavage medium (Cook, Brisbabe, Australia) in an atmosphere of 6 % CO_2, 5 % O_2 and 90 %N_2.

Sixteen of 31 activated oocytes were fertilized (51.6 %), and developed into well-cleaved embryos. One well-cleaved embryo (eight-cell stage) was selected and transferred on Day 3. After the embryo transfer, seven well-cleaved embryos were cryopreserved using the vitrification method [20]. Subsequently one gestational sac was identified on ultrasound. A healthy, 2750 g female infant (46, XX) was delivered at 40 weeks of gestation by caesarean section.

Discussion

Since the introduction of ICSI in the treatment of male infertility, modern sperm recovery techniques have made it possible to help men with obstructive azoospermia (OA) or nonobstructive azoospermia (NOA) to achieving fertilization in vitro. Especially, cryopreservation of testicular spermatozoa prior to ICSI is routinely performed in patients with OA and NOA [21], because if pregnancy is not achieved in the first TESE-ICSI cycle, a repeat TESE may be necessary. The results of TESE-ICSI are determined by the availability of motile spermatozoa and the type of azoospermia, either OA or NOA. ICSI with testicular spermatozoa from men with NOA results in lower fertilization and pregnancy rates compared with men with OA [22]. Motile spermatozoa, either from fresh or frozen TESE, are necessary for optimal fertilization and pregnancy outcomes. Frozen testicular spermatozoa further reduced the availability of motile spermatozoa [23]. Thus, most men with OA or NOA can be treated successfully with TESE-ICSI with the exception of some cases.

Artificial oocyte activation can be induced by a variety of electrical stimulation and chemical substances. The efficacy of chemical substances used after ICSI in couples who experienced complete fertilization failure or low fertilization rates in previous cycles of ICSI has been demonstrated [24]. Calcium ionophore treatment, in particular, is the most commonly applied method for oocyte activation in clinical trials; this causes a single transient increase in intracellular calcium (Ca^{2+}) in the oocyte. This function is called the "trigger" [25]. Subsequently, a transient increase in intracellular Ca^{2+} occurs after sperm-egg fusion, followed by calcium oscillation, which continues for 3–4 h [26]. Calcium oscillation during mitosis and the exit from meiosis increases the cell number of the inner cell mass in blastocysts. It has been shown that the calcium trigger and oscillation plays a significant role in embryo development. Successful first pregnancy and delivery have been reported as a result of calcium ionophore with ICSI using immobilized or motile spermatozoa [27]. In addition, combining

calcium ionophore treatment with ICSI suggested effectiveness in the surgical retrieved of spermatozoa [28]. In addition, several cases of successful pregnancy and delivery following ICSI of frozen-thawed nonviable testicular sperm with calcium ionophore have been reported [29, 30].

Recently, several reports have demonstrated the efficacy of $SrCl_2$ after ICSI in couples who experienced complete fertilization failure or low fertilization rates in previous cycles of ICSI [17–19]. These results show that $SrCl_2$ treatment is useful for activating human oocytes that frequently fail to fertilize in ICSI. In addition, calcium oscillation patterns with $SrCl_2$ treatment appear closer to the pattern which occurs with spontaneous fertilization than artificial activation by calcium ionophore [31]. However, it is not known whether or not $SrCl_2$ is as useful for oocyte activation as ICSI with frozen-thawed testicular spermatozoa. In this report, we decided to use $SrCl_2$ to activate the injected oocytes, because it is one of the most efficient agent for AOA. To our knowledge, this is the first clinical report of pregnancy and delivery following the transfer of embryo resulting from frozen-thawed testicular sperm injection and $SrCl_2$ oocyte activation. It is believed that AOA using $SrCI_2$ as a safe and effective method for fertilization of unfertilized oocytes. Although the safety of $SrCl_2$ treatment has been clarified, further studies are needed, because the long-term effects of ICSI with $SrCl_2$ on the resulting babies and children remain largely unknown.

In conclusion, we report the achievement of a live birth after $SrCl_2$ oocyte activation in previous repeated failed or low fertilization after ICSI of frozen-thawed testicular spermatozoa. However, the mechanism and genetic safety of oocyte activation induced by $SrCl_2$ treatment are not entirely clear. Therefore, further study and tests are required to confirm the safety of $SrCl_2$ treatment in oocyte activation for clinical application. Although it is safe according to the clinical outcomes thus far, patients must be informed regarding the potential risks of the prescribed fertility treatment and the possible long-term health implications for the child.

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