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Successful pregnancy and delivery after ICSI with artificial oocyte activation by calcium ionophore in in-vitro matured oocytes: a case report


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Abstract The achievement of a successful pregnancy and delivery after oocyte activation with calcium ionophore is reported in a couple having low fertilization rates after intracytoplasmic sperm injection (ICSI) of in-vitro matured oocytes. A couple, in which the wife had polycystic ovary syndrome and the husband had moderate oligoteratozoospermia, showed a low fertilization rate in a previous in-vitro maturation cycle (2/11 [18.2%]). The most likely cause of complete fertilization failure or low fertilization rates is failure of oocyte activation. Therefore, artificial oocyte activation by calcium ionophore was combined with ICSI to achieve viable fertilized oocytes. Oocytes were stimulated with calcium ionophore for 30 min after ICSI. The fertilization rate of oocytes activated with calcium ionophore (13/15 [86.7%] and 7/9 [77.8%]) was higher than that of the non-activated oocytes. In the latest cycle, three embryos derived from the activated oocytes were transferred into the uterus on day 3. Subsequently, two gestational sacs were identified on ultrasound. The patient delivered dizygotic twins (girl 2260 g and boy 2760 g) at 35 weeks and 6 days gestation by caesarean section. This result suggests that calcium ionophore could be useful for oocyte fertilization in couples with low fertilization rates after ICSI of in-vitro-matured oocytes. 

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KEYWORDS: Artificial oocyte activation, Calcium ionophore, Immature oocytes, IVF, PCOS

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Introduction

A number of disadvantages are associated with gonadotrophin use in ovarian stimulation for IVF cycles. These include the risk of ovarian hyperstimulation syndrome (OHSS) (Cobo et al., 1999), high drug costs, the need for daily injections and frequent monitoring. In-vitro maturation (IVM) of immature oocytes retrieved from unstimulated ovaries has the potential to avoid these problems. It has been reported that priming with HCG before immature oocyte retrieval in women with polycystic ovary syndrome improves the rate of oocyte maturation and pregnancy (Chian et al., 1999, 2000; Son et al., 2006), and, occasionally, results in obtainment of in-vivo matured oocytes at the time of retrieval (Son et al., 2008b).

Intracytoplasmic sperm injection (ICSI) is considered to be a powerful tool in assisted reproductive techniques. Although the fertilization rate with ICSI is typically considered to be the highest among the assisted reproductive techniques currently offered, the reported complete fertilization failure rate after ICSI ranges between 1.29% and 3% (Esfandiari et al., 2005; Liu et al., 1995). Complete fertilization failure or low fertilization rates after ICSI can be observed repeatedly in some couples. It is known that oocyte activation does not occur in about 70% of unfertilized oocytes after ICSI, despite accurate injection of the spermatozoon into the cytoplasm of the oocyte (Yanagida, 2004). The reasons for this occurrence may include failure of sperm head decondensation, premature sperm chromatin condensation, oocyte spindle defects and sperm aster defects (Swain and Pool, 2008). Furthermore, fertilization failure can occur as a result of limited availability of mature or morphologically normal oocytes, a lack of motile spermatozoa, and severe forms of teratozoospermia, such as globozoospermia (Dam et al., 2007; Yanagida, 2004). In women whose oocytes were not fertilized in previous IVF cycles, some methods of oocyte activation, such as electroporation (Mansour et al., 2009; Yanagida et al., 1999), or using chemical substances such as calcium ionophore (Ahmady and Michael, 2007; Borges et al., 2009; Chi et al., 2004; Eldar-Geva et al., 2003; Heindryckx et al., 2005; Kim et al., 2001; Kyono et al., 2009; Tejera et al., 2008; Terada et al., 2009; Tesarik and Sousa, 1995; Yoon et al., 2013), calcium ionophore and puromycin (Murase et al., 2004; Nakagawa et al., 2001), calcium chloride and ionophore (Rybouchkin et al., 1997), or ionomycin (Nasr-Esfahani et al., 2008), and strontium chloride (Kim et al., 2012, 2014) are reported to be effective in the formation of pronuclei and achieving childbirth. Specifically, calcium ionophore was found to be an excellent candidate for improving fertilization, embryo quality and pregnancy rates in women who showed complete fertilization failure or low fertilization rates (Chi et al., 2004; Eldar-Geva et al., 2003; Kyono et al., 2009; Terada et al., 2009; Tesarik and Sousa, 1995). Therefore, calcium ionophore is typically used clinically for artificial oocyte activation. To the best of knowledge, immature oocyte activation with calcium ionophore in the IVM cycle has not been reported.

In the present study, a case of successful pregnancy and delivery after artificial activation of IVM oocytes by calcium ionophore is reported.

Case report

A 32-year-old woman and her 33-year-old husband visited our clinic for primary infertility. The woman had irregular menstrual cycles and normal early follicular and mid-luteal phase serum hormone concentrations. A transvaginal ultrasound examination carried out during the early follicular phase established the diagnosis of PCOS. The diagnosis was based on Rotterdam consensus criteria (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Semen analyses showed semen volumes of 1.5–2.5 ml, sperm counts of $4-10 \times 10^6/\text{ml}$ with 53% motility, and 2–4% strict morphology. The couple had failed to achieve pregnancy after two cycles of intrauterine insemination and ovarian stimulation with clomiphene citrate and gonadotropins.

The initial IVM study was approved by the Institutional Review Board of Maria Fertility Hospital (IRB reference number 2008-001, approved 1 July 2008). Although ovarian stimulation for IVF was recommended to the couple, other treatment options, including artificial oocyte activation with calcium ionophore combined with IVM, were discussed. The couple chose to undergo an IVM cycle immediately. Subsequently, they opted for IVM treatment with calcium ionophore oocyte activation after first cycle. Because of the risk of OHSS, calcium ionophore oocyte activation has great potential, especially in patients showing compromised fertilization rates below 29% after ICSI in previous cycles (Montag et al., 2012). In the first IVM cycle; 15 germinal vesicle stage immature oocytes were retrieved, and 11 mature oocytes were obtained after day 1 culture. The other four immature oocytes were arrested on day 2; therefore, these oocytes were discarded. Fertilization rate was 18.2% (2/11). Embryonic development was assessed on day 3 of culture. Embryo quality was defined as follows: well-cleaved embryo (at least six or more blastomeres), less than 20% of anucleate fragments and no apparent morphological abnormalities; moderately cleaved embryo (at least six or more blastomeres), 20–50% of anucleate fragments and no apparent morphological abnormalities or an embryo that did not reach the six blastomeres but had less than 50% of anucleate fragments; poorly-cleaved embryo (embryos that were not well-cleaved or moderately cleaved) (Yoon et al., 2011). Two poorly cleaved embryos (five-, and two-cell stage) were transferred on day 3, but pregnancy was not achieved.

On day 3 of the second and third cycles, the woman underwent a baseline ultrasound scan; small follicles were seen in both ovaries (all less than 4 mm in diameter), confirming no dominant follicle. In the second cycle, the endometrial thickness was 7.9 mm in the ultrasound scan performed on day 8, and 10,000 IU of HCG (IVF-C; LG Life Science, Korea) was administered. Oocyte retrieval was carried out 38 h after HCG priming (Son et al., 2008a). Transvaginal ultrasound-guided oocyte collection was carried out using a 19-gauge aspiration needle (K-OPS-7035-RWH-ET; Cook, Australia) with a reduced aspiration pressure of 7.5 kPa. The aspirates were collected in tubes with pre-warmed heparinized saline. To avoid the possibility of missing oocytes with a small amount of cumulus cells, the follicular aspirates were filtered using a 70- μm mesh size (Falcon, Becton Dickinson, NJ). They were washed with oocyte wash medium (Biosupply Co., Korea) and oocytes were isolated under a stereomicroscope. A total of

20 GV-stage immature oocytes were obtained. These oocytes were cultured in IVM medium (Biosupply Co., Korea) supplemented with 40% of the patient's own serum (inactivated at 56°C for 30 min) and a final concentration of 0.6 IU/ml recombinant human FSH (Gonal-F; Merck Serono, Italy), 0.1 IU/ml human menopausal gonadotropin (Pergoveris; Merck Serono, Switzerland), and 10 ng/ml recombinant human epidermal growth factor (Gibco Invitrogen, Carlsbad, CA) at 37°C in an atmosphere containing 6% CO₂, 5% O₂, and 90% N₂. Eight and seven mature oocytes were obtained after day 1 and day 2 of culture, respectively. The other five immature oocytes were discarded. Semen was allowed to liquefy for 30 min at room temperature before collection of spermatozoa by the swim-up method using MRC#D01 medium (Fertilization medium; Biosupply Co., Korea). The mature oocytes were inseminated by ICSI according to their maturational status 24 h or 48 h after retrieval (eight on day 1, and seven on day 2). Within 30 min of injection, all of the oocytes were exposed to 10 μM of calcium ionophore A 23187 (Sigma, St Louis, MO) for 30 min (Rybouchkin et al., 1997; Tesarik et al., 2000). The oocytes were subsequently rinsed several times in MRC#D01 medium. Thirteen oocytes were fertilized after ICSI (86.7%). The zygotes were cultured in MRC medium series (Biosupply Co., Korea) (Yoon et al., 2011). Embryonic development was assessed on day 4 of culture. One well-cleaved embryo (eight-cell stage), one moderately cleaved embryo (eight-cell stage), and one poorly cleaved embryo (eight-cell stage) were selected and transferred at 4 days after oocyte retrieval. For the endometrial preparation, the patient received oestradiol valerate (Progynova; Bayer Schering Pharma AG, Germany), starting on the day of oocyte retrieval. As the endometrial thickness was 8 mm, a 6 mg dose was administered, all in divided doses following the protocol described by Lim et al. (2009). Luteal support was provided by administering 100 mg of progesterone (Watson Pharmaceuticals Inc., Corona, CA) daily starting on the day of initial ICSI. Pregnancy did not occur after embryo transfer.

Three months later, this couple again underwent the same procedure. In the third cycle, an endometrial thickness of 8.5 mm was noted. The patient was given 10,000 IU of HCG, and oocytes retrieval was carried out 38 h after HCG priming. At the time of retrieval, 12 oocytes were obtained, and one oocyte was in the mature form and the other 11 GV-stage immature oocytes were cultured in IVM medium. Eight mature oocytes were obtained after day 1 of culture. The other three immature oocytes were arrested on day 2; therefore, these oocytes were discarded. A total of nine mature oocytes, one from day 0, and eight from day 1, were inseminated by ICSI about 2–3 h and 24 h after retrieval, respectively, and all of the oocytes were exposed to calcium ionophore. Seven oocytes were fertilized after ICSI (77.8%). Embryonic development was assessed on day 3 of culture. One well-cleaved embryo (eight-cell stage), and two moderately-cleaved embryos (six-cell stage) were selected and transferred on day 3. At the time of embryo transfer, the endometrial thickness was 8.5 mm.

The serum beta-HCG concentration at 14 days after collection was 290 mIU/ml. Two weeks later, a twin clinical pregnancy with two fetal heartbeats was confirmed by transvaginal ultrasonography. Finally, healthy female (2260 g) and male (2760 g) babies were delivered at 35 weeks and 6 days gestation by caesarean section.

Discussion

The benefits of IVF for several types of infertility has been proven, and ICSI has enabled fertilization of oocytes from patients whose partners have extremely low numbers of viable sperm and a very low probability of achieving fertilization *in vitro*. Complete fertilization failure or low fertilization rates, however, are still observed in some cases, such as those of globozoospermia, teratozoospermia, immotile spermatozoa, and even unexplained cases. Most fertilization failures after ICSI reportedly manifest as non-activated oocytes. The failure of fertilization after ICSI may result from either the lack or deficiency of activation factors in spermatozoa, the inability of the oocyte to respond to the injected sperm, or the lack of ooplasmic factors triggering sperm chromatin decondensation (Nasr-Esfahani et al., 2010).

Artificial oocyte activation can be induced by electrical stimulation and a variety of chemical substances. The efficacy of chemical substances used after ICSI has been demonstrated in couples who experienced complete fertilization failure or low fertilization rates in previous cycles of ICSI (Chi et al., 2004; Eldar-Geva et al., 2003; Kim et al., 2012, 2014; Murase et al., 2004; Terada et al., 2009). Calcium ionophore treatment, in particular, is the most commonly used method for oocyte activation in clinical trials. Following sperm-oocyte fusion, a transient increase in intracellular calcium released from internal stores is the trigger, which is initiated by a receptor-mediated interaction between the spermatozoon and the oocyte plasma membrane. This increase in calcium ion (Ca²⁺) alone is insufficient to fully activate the oocyte and, 30 min later, it is followed by the second function, calcium oscillation (Swann and Ozil, 1994). The oscillator drives the release of calcium ions from intracellular stores in the oocyte and supports a series of shorter Ca²⁺ transients of high amplitude for 3–4 h, which is critical for complete oocyte activation (Tesarik et al., 2000). Activation of the oocyte results in the resumption of meiosis, extrusion of the second polar body, and the formation of pronuclei, and allows the oocyte to proceed to embryonic development (Stricker, 1999). It has been shown that the calcium trigger and oscillation play a significant role in embryo development, and pregnancy can be achieved.

In-vitro maturation of immature oocytes has been proposed as a potential alternative for conventional IVF treatment after ovarian stimulation. The number of babies resulting from IVM is increasing since the first report of successful pregnancy by Trounson et al. (1994). The collection of immature oocytes from unstimulated ovaries, followed by in-vitro oocyte maturation and subsequent insemination, is particularly beneficial for women who are at risk of developing OHSS (Chian et al., 1999, 2000). Recently, it has been reported that priming with FSH or HCG before immature oocyte retrieval improved oocyte maturation rates as well as pregnancy rates in women with PCOS (Chian et al., 1999, 2000). In HCG-primed IVM cycles, the expansion of cumulus cells around the oocytes caused by a high dosage of HCG (10,000 IU) facilitates detachment and expulsion of the cumulus oocyte complex mass from the follicle during the aspiration, and therefore, it become easy to perform oocyte collection as well as oocyte identification (Son et al., 2006). In general, however, fertilization, implantation and childbirth rates with IVM are

still lower than those achieved with conventional IVF (Son et al., 2008b). A number of factors might lead to lower implantation and childbirth rates with IVM, including non-synchronization of oocyte nuclear and cytoplasmic maturation, culture conditions, and timing of insemination. Especially, non-synchronization of oocyte nuclear and cytoplasmic maturation was considered to be a major reason for poor developmental competence of IVM oocytes (Combelles et al., 2002; Moor et al., 1998). Chian et al. (2000) reported that HCG administration *in vivo* can hasten the nuclear maturation process, whereas its effect on cytoplasmic maturation is unclear. In addition, one of the important factors regulating the number and quality of oocytes maturing *in vitro* are the culture conditions and timing of insemination used for IVM.

In the present study, although no problems occurred in the semen analysis, the possibility that the male had a defective sperm-associated oocyte activating factor that resulted in oocyte activation failure cannot be ruled out. Moreover, we could not identify whether a sperm- and oocyte-related deficiency existed in the couple (Heindryckx et al., 2005), because heterologous ICSI had not been performed owing to ethical issues. In our study, the average maturation rate of IVM oocytes was 73.9% (34/46). The average maturation rates of IVM oocytes at 24 h and 48 h were 58.7% (27/46) and 15.2% (7/46), respectively. At our IVF centre, the overall average maturation rate of IVM oocytes was 65.7% and the rates at 24 h and 48 h were 55.8% and 9.9%, respectively (unpublished data). A fertilization rate of 83.3% (20/24) was achieved after the calcium ionophore treatment; whereas in the previous (non-activated) cycle, a fertilization rate of only 18.2% (2/11) was achieved. Furthermore, insemination was carried out at the optimal time reported previously (Hyun et al., 2007).

Many cases of successful pregnancies have occurred after calcium ionophore oocyte activation in patients with complete fertilization failure or low fertilization rates after conventional IVF (Nasr-Esfahani et al., 2010). To date, pregnancy and delivery after transfer of embryos produced from IVM oocytes after artificial activation with calcium ionophore has not been reported. In the present study, a case of successful pregnancy and delivery after artificial oocyte activation with calcium ionophore is reported low in a couple with low fertilization rates despite ICSI of previous IVM oocytes.

In HCG-primed IVM cycles, a few *in-vivo* matured oocytes can be obtained at the time of collection (Son et al., 2006, 2008a, 2008b). These *in-vivo* matured oocytes produced better-quality embryos than those derived from *in-vitro* matured oocytes, resulting in better clinical pregnancy rates (Son et al., 2008a, 2008b). The nuclear maturation of oocytes was also enhanced, although the underlying mechanism remained unclear (Chian et al., 2000). In the third cycle, the patient had mixed embryos, with one embryo produced from the *in-vivo* matured oocyte and two embryos produced from the *in-vitro* matured oocytes, and she had a twin pregnancy. This result indicates that the embryos generated from sibling immature oocytes have implantation potential. Therefore, this study showed that sibling immature oocytes might not be affected detrimentally if a matured oocyte is present at oocyte collection (Son et al., 2008b). Although the effect of HCG priming on oocyte maturation and developmental competence in IVM cycles was maintained, further studies are needed because the mechanism is still unclear.

In conclusion, this case report demonstrates that a clinical pregnancy and delivery was achieved after artificial activation of mature oocytes with calcium ionophore in IVM cycles after HCG priming. It is an effective option, especially in couples with low fertilization rates in IVM cycles as well as in IVF cycles.

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