

Live Birth after *In Vitro* Maturation of Oocytes in a Patient with Repeated Fertilization Failure in IVF: A Case Report

Smirnova A^{1,2}, Anshina M^{1,2}, Sergeev S^{1,2} and Ellenbogen A^{3,4*}

¹IVF and Genetics Center "FertiMed", Moscow, Russia

²Department of Endocrinology, Russian University of People's Friendship, Moscow, Russia

³Department of Obstetrics and Gynecology, IVF Unit, Hillel Yaffe Medical Center, Hadera, Israel

⁴Rappaport School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

*Corresponding author: Ellenbogen A, School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, Tel: 972506246722; Fax: 97299512184; E-mail: ellenbogen55@gmail.com

Rec date: June 07, 2016; Acc date: June 28, 2016; Pub date: June 30, 2016

Copyright: © 2016 Smirnova A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

The incidence of fertilization failure has decreased dramatically since the intracytoplasmic sperm injection procedure (ICSI) was successfully introduced into clinical practice. However, even in very successful units with remarkable pregnancy rates, there are couples who face repeated *in vitro* fertilization (IVF) failure due to low or absence of fertilization. We present a case of a 36-year-old woman with tubal factor of infertility and two previous IVF attempts with short and long agonist protocols and absence of normal fertilization after ICSI. The third cycle performed with *in vitro* maturation of oocytes (IVM) led to the development of top quality embryos. Two transferred embryos ensued in a singleton pregnancy and term delivery of a healthy girl.

Keywords: *In vitro* maturation; Oocyte; Fertilization failure; IVF

Introduction

The *in vitro* fertilization and embryo transfer technique was introduced into routine clinical practice more than thirty-five years ago and enables overcoming almost all types of infertility. Currently, more than five million children have been born due to this treatment. Nevertheless, not every IVF attempt leads to childbirth. Most cases of IVF failure can be explained by genetic defects or poor developmental potential of the embryo or low receptivity of the endometrium, which leads to implantation failure. A less frequent reason is fertilization failure. The precise cause of this result is not fully understood. A multiple regressive analysis performed in 304 couples with fertilization failure and 304 controls revealed the following risk factors: female smoking, non-tubal factor of infertility, a low count of progressive motile spermatozoa after preparation and less than four oocytes retrieved [1]. Implementation of the ICSI technique into clinical practice resolved most problems associated with low fertilization rate due to male factor: insufficient acrosome reaction, impaired binding with zona pellucida, very low progressive motility and abnormal sperm morphology. Nevertheless, in 1-3% of the IVF population all retrieved oocytes showed no sign of fertilization even after ICSI. Sixteen cases of total fertilization failure among 3723 ICSI cycles was reported (4.3%). In 10 cases, the next attempts at ICSI also resulted in total fertilization failure [2].

A recently published meta-analysis regarding fertilization rates after ICSI in patients with unexplained infertility, revealed that the probability of normal fertilization after ICSI in these patients is higher than after IVF (1.35-1.65, RR 1.49), and the risk of total fertilization failure after IVF is 8.2 times higher compared to ICSI [3]. On the other hand, it was suggested that total fertilization failure is not related to

sperm parameters but rather as a result of suboptimal response to ovarian stimulation [4] or oocyte post maturity [5].

Alternative approaches to overcoming repeated fertilization failure after ICSI, are oocyte or sperm donation, artificial oocyte activation [6,7] and *in vitro* maturation of oocytes.

We present our experience of infertility treatment in women with tubal infertility, diminished ovarian reserve and total fertilization failure after two attempts of ICSI.

Case Report

A 36-year-old woman was referred to the IVF and Reproductive Genetics Center in December 2013 for infertility treatment. She had been pregnant three times. Her first pregnancy, in 1997, resulted in spontaneous preterm delivery at 30 weeks. The newborn girl died two months after delivery. Her second and third pregnancies were ectopic: in 2005 she underwent laparotomy and right salpingectomy and in 2012 laparoscopic left salpingectomy.

Her partner was 41-years-old and had two children from a previous marriage. Sperm analysis showed normal zoospermia. The patient had a regular menstrual cycle of 26-27 days. Her hormonal profile on day 3 of cycle was: AMH 0.5 ng/ml, FSH 11.8 IU/l, LH 4.7 IU/l, 17 β estradiol 75 pg/ml. An ultrasound scan on day 3 of menses revealed a normal size uterus, a right ovary 24 \times 14 \times 16 mm, containing 3 antral follicles 4-7 mm in diameter, and a left ovary 27 \times 13 \times 15 mm, with 3 antral follicles 5-8 mm in diameter.

The first IVF attempt was performed in January 2014, using a short agonist protocol with triptorelin (Decapeptyl, Ferring GmbH, Kiel, Germany) 0.05 mg/daily, starting from day 2 of cycle and follitropin alpha + lutropin (Pergoveris, Merck-Serono C.A., Switzerland) 300 IU daily, for 14 days starting from day 2 of cycle. Due to slow follicle

growth, menopropin (Merional, IBSA Institut Biochimique SA, Switzerland) 150 IU daily, was added from day 7 of stimulation. Human chorionic gonadotropin (hCG) (Pregnyl, N.B. Organon, Oss, Netherland) 10,000 IU was administered intramuscularly on day 15 of stimulation when two leading follicles achieved a diameter of 18 mm. Transvaginal follicle aspiration under ultrasound guidance and general sedation was performed 35 hours after triggering and 5 MII oocytes were retrieved. Sperm count on the day of ovum pick up demonstrated volume 2.3 ml, concentration 122×10^6 per ml, motility (a +b) 61%, and normal morphology. Because few oocytes were retrieved, we considered fertilization by ICSI. Briefly, 2 μ l of sperm suspension was pipetted to the center of a microdroplet (5-7 μ l) of polyvinylpyrrolidone (PVP; SAGE media, Cooper Surgical, Inc., USA.). Spermatozoa reaching the microdroplet edge were collected with a finely drawn ICSI pipette (Origio Inc., USA), transferred to a clean droplet of PVP for tail breaking, and injected into oocytes one hour after denudation [8]. Nineteen hours after routine ICSI, one oocyte developed in 3 pn and the other 4 oocytes degenerated.

A second IVF attempt was performed in April 2014, with a long agonist protocol. Triptorelin 0.05 mg/daily was administered from the mid luteal phase and menopropin 450 IU daily was started when ovarian suppression was achieved and continued for 11 days. Human chorionic gonadotropin 10,000 IU was administered intramuscularly on day 14 of stimulation, when three leading follicles achieved a diameter of 20 mm and another five follicles were 12 to 18 mm in diameter.

Four follicles >18 mm were aspirated 35 hours after hCG triggering, and no oocytes were retrieved. A second aspiration of the other 4 follicles >17 mm that developed was performed the next day, with 4 oocytes being obtained: 2 MII, 1 MI and 1 GV. Sperm count demonstrated volume 2 ml, concentration 78×10^6 per ml, motility of 56%, and 4% normal morphology. No normal fertilization occurred 24 hours after ICSI. Taking into consideration the possibility of poor oocyte quality obtained due to the protocols that had been used, we decided to change the stimulation protocol. A third stimulation was done with clomiphene citrate, 50 mg daily from day 3 to day 7 of cycle, and menopropin 75 IU daily was started on day 7 of stimulation. A single follicle <15 mm developed and the cycle was cancelled due to poor response of stimulation.

Taking into consideration the repeated fertilization failure in conventional IVF attempts, IVM+IVF was performed in August 2014 with FSH priming (Merional 150 IU on day 2, 3 and 4 of cycle), hCG 10,000 IU was given as a trigger on day 7 of the cycle. Six follicles 5-12 mm in diameter were aspirated under ultrasound guidance using a single channel needle 17/20 G (Swemed Sense, Vitrolife, Sweden) and reduced aspiration pressure of 7.5 Pka, 39 hours after triggering. Five oocytes were obtained. All oocytes were cultured for 5.5 hours in maturation medium (SAGE IVM media kit, Cooper Surgical Company, USA) supplemented with FSH + LH (Merional, IBSA Institut Biochimique S.A., Switzerland) for a final concentration of 75 mIU/ml. The retrieved oocytes were stripped for maturity assessment 5 hours after aspiration as described previously [9]. Four oocytes were MII and one was GV. All MII oocytes were fertilized by ICSI on the day of aspiration, three oocytes developed in 2 PM, 18 hours after ICSI, and one oocyte degenerated. Three top quality embryos (10-cells grade 1) were obtained. Two of them were transferred to the patient and one was vitrified 72 hours after fertilization. Luteal phase support was initiated with estradiol valerate (Proginova, Shering, France) 2 mg 3 times a day, starting from the day of follicle aspiration, and

progesterone (Utrogestan, Besins Healthcare, Belgium) 200 mg 3 times a day was vaginally started the day after ICSI was performed. A singleton ongoing pregnancy emerged as diagnosed by ultrasound scan six weeks after the embryo transfer. A cesarean section was performed at 39 weeks of pregnancy, and a healthy girl was born.

Discussion

The causes of repeated fertilization failure are diverse. They include patients who have undergone repeated IVF cycles with low-quality embryos, maturation arrest of human oocytes [10] and a subgroup of patients with the unclear diagnosis of egg factor. In some cases, no eggs are retrieved in spite of apparently normal follicular development, a situation also known as the controversial empty follicle syndrome. Another possibility of fertilization failure may be the result of suboptimal response to ovarian stimulation. Five-hundred fifty-five couples who had total fertilization failure during conventional cycle of IVF or ICSI cycles were assessed [4]. Delivery rates for IVF patients who elected to continue treatment after fertilization failure were 44% per patient, 25% per embryo transfer (ET), and 22% per cycle. Delivery rates for ICSI patients were 36% per patient, 23% per ET, and 18% per cycle. The number of mature oocytes obtained was always statistically significantly lower in the total fertilization failure cycle when compared with fertilization cycles, whether ICSI or conventional IVF was involved. This report suggests that prognosis for pregnancy is encouraging in subsequent cycles after total fertilization failure as a result of suboptimal response to ovarian stimulation that can be corrected. Different modifications of ovarian stimulation protocols were proposed to improve oocyte maturation: extending the *in vivo* period of follicular growth to a follicular size of >20 mm, increasing the triggering hCG dose, GnRH agonist administration as a trigger of final maturation, prolonging the period between triggering and follicle aspiration from 35-36 to 40 hours, or double triggering [11-13]. A natural cycle protocol has been performed in some cases as well, with disappointing results. As a different approach from the above, *in vitro* maturation is a promising technology in the field of human infertility [14]. In addition to indications for treating patients with polycystic ovarian syndrome or for preserving fertility, IVM was proposed for treating patients with poor ovarian response and it may serve the last choice of treatment after the failure to achieve pregnancy in traditional IVF [15]. It has also been proposed for use in rare conditions such as rescue of oocytes which have failed to mature in stimulated cycles [16] or in cases with unexplained primarily poor quality embryos. A recent study examined the efficacy of IVM in seven patients with three or more IVF failures due to abnormal oocyte development due to empty follicle syndrome, oocyte maturation arrest, or failure of fertilization. Four women received minimal ovarian stimulation with FSH. Oocytes were obtained in all patients, mean maturation rate was 39.6% and mean fertilization rate was 45.8%. Embryo transfer was performed in four women; and two patients with previous empty follicle syndrome conceived and delivered [17].

We have presented an additional rare case with repeated fertilization failure due to egg maturation abnormalities. The IVM cycle performed obtained four mature oocytes fertilized by ICSI. Transfer of two embryos resulted in a successful pregnancy. It could be possible that substitution of the natural milieu with maturation of the oocyte *in vitro* might be beneficial in those cases. In conclusion, IVM of oocytes should be considered as a beneficial option in patients with repeated fertilization failure.

References

1. Krog M, Prior M, Carlsen E, Loft A, Forman J, et al. (2015) Fertilization failure after IVF in 304 couples-A case-control study on predictors and long-term prognosis. *Eur J Obstet Gynecol Reprod Biol* 184: 32-37.
2. Shinar S, Almog B, Levin I, Swartz T, Amit A, et al. (2014) Total fertilization failure in intra-cytoplasmic sperm injection cycles--classification and management. *Gynecol Endocrinol* 30: 593-596.
3. Johnson L, Sasson I, Sammel M, Dokras A (2013) Does intracytoplasmic sperm injection improve the fertilization rate and decrease the total fertilization failure rate in couples with well-defined unexplained infertility? A systematic review and meta-analysis. *Fertil Steril* 100: 704-711.
4. Kinzer D, Barrett C, Powers D (2008) Prognosis for clinical pregnancy and delivery after total fertilization failure during conventional in vitro fertilization or intracytoplasmic sperm injection. *Fertil Steril* 90: 284-288.
5. Goud P, Goud A, Oostveldt VP, Elst VJ, Dhont M (1999) Fertilization abnormalities and pronucleus size asynchrony after intracytoplasmic sperm injection are related to oocyte postmaturity. *Fertil Steril* 72: 245-252.
6. Meerschaut VF, Nikiforaki D, Heindryckx B, Sutter DP (2014) Assisted oocyte activation following ICSI fertilization failure. *Reprod Biomed Online* 28: 560-571.
7. Montag M, Koster M, Van VK, Bohlen U, Ven HV (2012) The benefit of artificial oocyte activation is dependent on the fertilization rate in a previous treatment cycle. *Reprod Biomed Online* 24: 521-526.
8. Cayan S, Lee D, Conaghan J, Givens CA, Ryan IP, et al. (2001) A comparison of ICSI outcomes with fresh and cryopreserved epididymal spermatozoa from the same couples. *Hum Reprod* 16: 495-499.
9. Shavit T, Ellenbogen A, Michaeli M, Kartchovsky E, Ruzov O, et al. (2014) In-vitro maturation of oocytes vs in-vitro fertilization with a gonadotropin-releasing hormone antagonist for women with polycystic ovarian syndrome: can superiority be defined? *Eur J Obstet Gynecol Reprod Biol* 179: 46-50.
10. Levran D, Farhi J, Nahum H, Glezerman M, Weissman A (2002) Maturation arrest of human oocytes as a cause of infertility: case report. *Hum Reprod* 17: 1604-1609.
11. Wang W, Zhang XH, Wang WH, Liu YL, Zhao LH, et al. (2011) The time interval between hCG priming and oocyte retrieval in ART program: a meta-analysis. *J Assist Reprod Genet* 28: 901-910.
12. Griffin D, Feinn R, Engmann L, Nulsen J, Budinez T, et al. (2014) Dual trigger with gonadotropin-releasing hormone agonist and standard dose human chorionic gonadotropin to improve oocyte maturity rates. *Fertil Steril* 102: 405-409.
13. Humaidan P, Alsbjerg B (2014) GnRH-a trigger for final oocyte maturation: is HCG trigger history? *Reprod Biomed Online* 29: 274-280.
14. Ellenbogen A, Shavit T, Shalom-Paz E (2014) IVM results are comparable and may have advantages over standard IVF. *FV&V in Obs Gyn* 6: 77-80.
15. Liu J, Lu G, Qian Y, Mao Y, Ding W (2013) Pregnancies and births achieved from in vitro matured oocytes retrieved from poor responders undergoing stimulation in in vitro fertilization cycles. *Fertil Steril* 80: 447-449.
16. Tan SL, Child TJ (2002) In-vitro maturation of oocytes from unstimulated polycystic ovaries. *Reprod Biomed Online* 4: 18-23.
17. Hourvitz A, Maman E, Brengauz M, Machtinger R, Dor J (2010) In vitro maturation for patients with repeated in vitro fertilization failure due to "oocyte maturation abnormalities." *Fertil Steril* 94: 496-501.