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Live birth following a single oocyte fertilized with ICSI and embryo transfer on day 2: a case report

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Short Communication

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Summary

In this report, we present a case of a couple who obtained a live birth with a single oocyte fertilized by intracytoplasmic sperm injection. The oocyte was collected at 36 h post trigger and was found to be at metaphase II when sperm injection was performed. At 18 h post injection, the oocyte was found to be fertilized with two clear pronuclei. The embryo divided and generated a four-cell embryo on day 2, which was replaced to the uterine cavity. Pregnancy test gave a positive β-human chorionic gonadotropin result, the scan performed at 7 weeks, revealed the presence of one amniotic sac with fetal heartbeat. Healthy live birth was obtained after 39 weeks of gestation.

Introduction

Over 8 million *in vitro* fertilization (IVF) children have been born since 1978 when the first IVF baby was announced (Steptoe and Edwards,1978). The number of couples facing infertility has increased steadily, many of whom will ultimately need assisted reproductive technology (Thoma *et al.*, 2013). Worldwide, approximately 2.5 million medically assisted reproduction (MAR) cycles are performed, resulting in over 500,000 deliveries annually. In the UK, IVF babies accounted for about 3% of all babies born in 2016 (Human Fertilisation and Embryology Authority, 2018; De Geyter *et al.*, 2018). IVF is a sophisticated process and, during the last 40 years, has improved steadily, therefore providing better a pregnancy chance for infertile couples. However, *in vitro* development of the human embryo is still suboptimal and many good quality embryos fail to implant and generate a viable pregnancy (Hu *et al.*, 1998; Zhao *et al.*, 2011; Niederberger *et al.*, 2018). The introduction of intracytoplasmic sperm injection (ICSI; Palermo *et al.*, 1992) has changed the treatment of male infertility, in particular for patients with a very low sperm count. Indeed, only a single spermatozoon would be needed to fertilize an oocyte and generate a successful pregnancy using the ICSI procedure. However, the number of oocytes collected is a major determinant of IVF success, with higher yields for better outcomes (Esteves *et al.*, 2019a). Controlled ovarian stimulation (COS) using exogenous gonadotropins to stimulate the ovary is applied to promote multifollicular development with the aim of harvesting multiple oocytes, which are collected surgically. Typically, a pharmacological dose of follicle stimulating (FSH) is used to induce the growth of multiple ovarian follicles. As follicles grow, luteinizing hormone (LH) exposure is provided to simulate the mid-cycle LH surge, which induces the processes of oocyte maturation and subsequent ovulation. Oocyte collection is therefore precisely timed to retrieve mature oocytes before the occurrence of ovulation. LH exposure initiates the resumption of meiosis and the maturation of the oocyte from the immature 'metaphase I' (MI) stage to the mature 'metaphase II' (MII) stage of development (Voronina and Wessel, 2003). Following ovulation, the remainder of the follicle forms the corpus luteum, which produces sex steroids, particularly progesterone, to prepare the endometrium for the implantation process of the embryo (Palomba *et al.*, 2015). Given the expected number of low oocyte in patients with reduced ovarian reserve and advanced maternal age (AMA), several strategies have been applied to raise the follicle number and oocyte collected, including the increased gonadotropin dose. The classic treatment remains the COS using the long GnRH agonist protocol, associated with hCG trigger for oocyte maturation (Esteves *et al.*, 2020). Despite that, in some patients undergoing IVF, occasionally only a limited number of oocytes are retrieved after COS. In this case, it is known that the likelihood of having a fertilized oocyte and a further transferable embryo with a successful pregnancy is very low, especially in women with AMA.

Case presentation

A married couple was referred to the reproductive medicine department at Royale Hayat Hospital, Kuwait City in 2019 for treatment. They presented with a 4-year history of primary

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infertility due to low ovarian reserve. The husband's age was 40 years, and semen analysis showed moderate asthenozoospermia. His wife was a 39-year-old woman with regular ovulatory cycles and FSH levels of 9.6 mIU/ml, anti-Müllerian hormone (AMH) 0.1 ng/ml and body mass index (BMI) 29. They underwent a full ICSI cycle at Royale Hayat Hospital. The female patient responded quite poorly at the COS. Briefly, 300 IU daily of recombinant human gonadotropin (Gonal-F, Merck, Rome, Italy) were administrated for 9 days (total gonadotrophin dose of 2700 IU). One dominant follicle was seen on the right ovary >18 mm, and another follicle was seen on the left ovary (15 mm). Oocyte maturation was triggered using a single injection of 5000 IU IVF-C (Human Chorionic Gonadotropin, Ciplamed) subcutaneously. Transvaginal ultrasound-guided oocyte retrieval was carried out 36 h after the trigger administration. Only one oocyte was retrieved during oocyte pick-up. At the egg collection day, the sperm count was 85×10^6/ml, the total motility was 13%, and the strict morphology 5%. We counselled the couple regarding the need for ICSI treatment given the low oocyte number and the couple agreed to this procedure.

Oocyte retrieval, ICSI, embryo culture and transfer

The cumulus–oocyte complex (COC) was isolated from follicular fluid and then rinsed in 1.0 ml G-MOPS™ plus medium (VitroLife, Göteborg, Sweden). Following the oocyte pick-up, the oocyte was transferred to 1.0 ml equilibrated G-IVF™ medium (VitroLife) at 37°C and 6% CO_2, 5% O_2 and nitrogen balance in a K-System incubator (K-System G210, CooperSurgical, Inc., USA) until the ICSI time. Sperm used for ICSI procedure was collected by masturbation and processed using a standard method as described by Bourne and colleagues (2004). Approximately 38–39 h after trigger, the oocyte was treated by hyaluronidase (80 mIU/ml) for 45–60 s to remove the surrounding cumulus cells. At that time, the oocyte was found to be at the MII stage, with clear extrusion of the first polar body. A single spermatozoon with normal morphology and progressive motility was selected under an inverted microscope (Nikon Eclipse Ti-S, Japan) and microinjected with the use of electrohydraulic injectors (Narishige, Japan). The oocyte was kept still by using a holding micropipette at the 9 o'clock position, and the polar body was oriented at the 12 o'clock position. The injecting pipette then was gently advanced through the zona pellucida and oolemma until the pipette was beyond the centre of the oocyte; then, the sperm was gently deposited into the oocyte's cytoplasm. The oocyte was examined for the presence of two pronuclei, and successful fertilization was confirmed at approximately 18 h after insemination. On day 2, the embryo was four-cell grade one, and was transferred to the uterine cavity (Figure 1). The embryo culture was completed, adopting sequential pre-equilibrated medium (VitroLife, G-series) as follows. Firstly, the fertilized oocyte was placed into a 20-µl drop of G-1™ medium covered by mineral oil. On the morning of day 2, the embryo was transferred from the G-1™ microdroplet to a µl droplet of EmbryoGlue (VitroLife) medium and kept in culture for almost 30 min, when embryo transfer was performed. We report here a compelling case in which a live birth was obtained from the only oocyte collected after COS. The embryo generated by ICSI was transferred on day 2 (Figure 1). The embryo replacement was completed under transabdominal ultrasound guidance using a soft transfer catheter (Wallace® Classic, CooperSurgical, USA). Luteal phase support with 90 mg progesterone vaginal gel twice a day (Crinone 8%, Merck) was started on the evening of egg collection

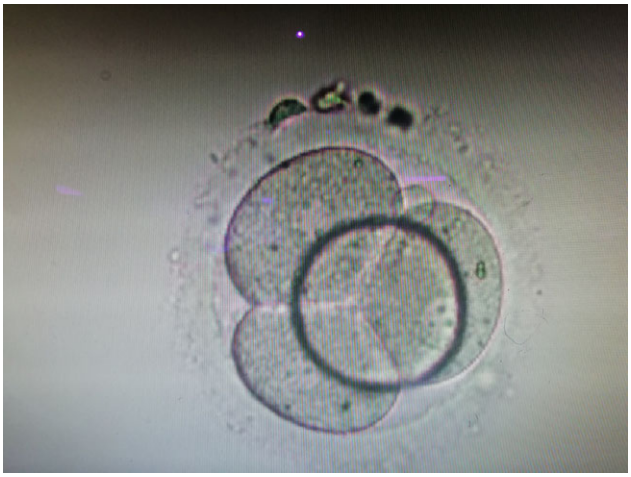

Figure 1. Embryo replaced on day 2 (four cells).

day and continued until the 9th gestation week add Progyluton tablet 2 mg, Clexane 0.4 ml and a tablet of baby Aspirin 75 mg daily. At the embryo transfer the patient started to take two injections weekly of Biosterone-Depot 250 mg and continued until 9 weeks of gestation.

Pregnancy outcome

The embryo implanted and gave a positive pregnancy with fetal heart, which resulted in a live birth female baby, born by caesarean section after 39 weeks of gestation.

Discussion

MAR outcome is dependent on multiple factors, including oocyte and sperm quality, maternal age, infertility cause, lifestyle factors, as well as laboratory conditions for manipulation and embryo culture (Gardner and Schoolcraft, 1999; Gardner; 2016; Sciorio and Smith, 2019). Appropriate oocyte cytoplasmic and nuclear maturation are paramount to ensure an optimal embryonic developmental competence. While nuclear maturation is usually obtained by the time of oocyte retrieval, cytoplasmic maturation cannot be assessed precisely, and it might negatively effect pregnancy outcome. However, an important aspect to mention is the importance of the number of oocytes retrieved in IVF patients, which has a direct effect on the outcome of MAR treatment, especially in patients with AMA (Esteves *et al.*, 2019a; Vaiarelli *et al.*, 2018). A recent paper published by Ubaldi and co-workers summarized the current and novel approaches for AMA patients undergoing IVF (Ubaldi *et al.*, 2019). This aspect has been investigated also in patients underwent to oocyte vitrification by Cobo and collaborators. The authors analyzed more than 6000 vitrified-warmed cycles, and reported an increased cumulative live birth rate from 15.8% with five oocytes to 32.0% with eight oocytes frozen. For younger patients (≤35 years old) 10 or 15 oocytes provided success rates of 42.8% and 69.8%. The highest cumulative live birth rate of 94.4% was obtained in younger patients when number of oocytes vitrified was 24 (Cobo *et al.*, 2018). With fresh oocytes, another study evaluated the minimum number of mature oocytes to achieve at least one euploid blastocyst for transfer. The study found that the age of the woman was the most critical predictor for the likelihood of achieving one euploid blastocyst. Based on this model a patient of 37 years old undergoing MAR treatment using

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ejaculated sperm needs between 9–13 mature oocytes to obtain at least one euploid blastocyst to transfer (Esteves *et al.*, 2019b). Therefore, in this case report, we were somewhat amazed to see that the only oocyte collected from a 39-year-old woman, was found to be mature and injected, fertilized and developed into a good quality embryo on day 2, when the embryo was transfer to the uterine cavity, and resulted in a viable pregnancy. Generally, the probability of having a good quality embryo to transfer after the retrieval of a single COC is low, and even lower in women with AMA. However, several elements, including maternal age, infertility cause, sperm parameters, and laboratory conditions such as temperature, pH, CO_2 and O_2 concentration (Gardner, 2016; Sciorio and Smith, 2019) during the embryo culture might influence both the fertilization and the embryo quality. This case's unique characteristic highlights the importance of MAR to overcoming the most adverse situations. Health professionals providing care to patients who are infertile should be aware that it is possible to provide couples like the one described here a real hope of biological parenthood. Equally important is to share such cases within the MAR community, which is often sceptical about the likelihood of one fertilized oocyte to generate a viable embryo for transfer, and ultimately a viable pregnancy with live birth.

Conclusion

We report here a compelling case in which a 39-year-old woman after COS was able to produce two follicles. Approximately 36 h after trigger, one oocyte was collected and then fertilized by ICSI. The fertilized embryo on day 2 was found to be at the four-cell stage, it was replaced to the uterine cavity, and resulted in a live birth female baby, born by caesarean section after 39 weeks of gestation.

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