Live birth of twins derived from zona-free oocytes

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Objective: To report a live birth of twins from autologous zona-free oocytes.
Design: Case report.
Setting: Reproductive endocrinology and infertility private practice and ambulatory in vitro fertilization (IVF) center.
Patient(s): A 34-year-old woman, gravida 0, with 100% zona-free oocytes.
Intervention(s): IVF with intracytoplasmic sperm injection (ICSI), blastocyst culture, and fresh embryo transfer.
Main Outcome Measure(s): Fertilization, blastocyst development, and live birth.
Result(s): A 34-year-old woman, gravida 0, conceived through IVF using autologous zona-free oocytes. The 11 retrieved oocytes were zona-free, from which eight were inseminated with ICSI; two embryos were transferred at morula and blastocyst stage, resulting in a twin pregnancy delivered at an estimated the gestational age of 37 weeks and 1 day.
Conclusion(s): Patients with the rare condition of 100% zona-free oocytes maintain the potential for pregnancy after careful micromanipulation of the oocytes. Caution is recommended on the number of embryos selected for transfer to reduce the risk of multiple gestation. (Fertil Steril® 2016;105:1232–5. ©2016 by American Society for Reproductive Medicine.)
Key Words: ICSI, in vitro fertilization, live birth, pregnancy, zona-free blastocyst, zona-free embryo culture, zona-free oocytes

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In human in-vitro fertilization (IVF) laboratories, zona-free oocytes (ZFOs), either encountered at the time of oocyte retrieval, at manipulation, or during culture are frequently observed (1–4). The majority of these ZFOs are the result of damage during manipulation rather than abnormal zona production. In 1999, Ding et al. (1) reported two cases of fertilization achieved using ZFO that had resulted from manipulation during cumulus removal in hyaluronidase solution (1). Shu et al. (2) reported two cases of successful vitrified-warmed embryos derived from ZFO that had resulted from handling during cumulus removal. Hiroaka et al. (3) described a successful pregnancy after vitrification of a human hatched blastocyst during culture. A pregnancy by intracytoplasmic sperm injection (ICSI) in a couple whose oocytes were zona-free due to abnormal zona production was reported by Stranger et al. (4).

Normally ZFOs are enclosed with corona or cumulus cells at the time of oocyte retrieval. The status of ZFOs is generally revealed after a stripping process for either fertilization assessment or ICSI. All ZFOs are typically discarded because they comprise a small percentage of oocytes retrieved. However, manipulation of the oocytes and fertilization are problematic for the embryology team when all oocytes are zona-free. Most ZFOs are easily damaged by the stripping process, so the embryo transfer is typically cancelled due to failed fertilization. The ZFOs that survive the stripping procedure are normally cultured to act as reserves in case no zona-intact embryos are available for transfer; in such cases, the zona-free embryos are normally frozen at the blastocyst stage. The first live birth from ZFO was reported in 2010 (2) after the transfer of vitrified-warmed blastocyst derived from a zona- and corona-cell-free oocyte. We report a case of complete ZFO due to abnormal zona production, which resulted in successful fertilization with ICSI, blastocyst transfer, and a term live birth of twins.

MATERIALS AND METHODS

A 34-year-old woman presented to our clinic with 2 years of male factor infertility due to severe oligozoospermia, and the consent from the patient for the case report has been obtained and is on file. Several months before presentation she had undergone an IVF cycle in which the six retrieved oocytes all had no zona pellucida, resulting in

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failed fertilization. At our center she underwent a repeat IVF cycle.

After 9 days of ovarian hyperstimulation using 5 mg of letrozole for 5 days and 300 IU of subcutaneous daily follicle-stimulating hormone, her peak estradiol concentration was 1,018 pg/mL, and she received 10,000 IU intramuscular human chorionic gonadotropin to trigger the final oocyte maturation. On the day of the oocyte retrieval, her partner's sperm sample had a concentration of 1.0×10^6 spermatozoa/mL with 40% motility and was washed twice with sperm wash medium (In Vitro Care) by centrifugation at $300 \times g$.

At egg retrieval, a total of 11 oocytes were obtained with cumulus complexes. Given her history of ZFO, all the patient's oocytes were initially assessed by a spreading technique in a drop of culture medium in a separate dish to facilitate viewing of the zona. Eight of the 11 oocytes appeared mature with a distant polar body and without zona. Three of the 11 oocytes were degenerated or empty cumulus complex, and no zona was observed. The eight matured oocytes determined suitable for insemination were divided into two groups to prepare for ICSI employing two different methods.

Four oocytes (group 1) were stripped successfully by passing through a fire-polished 5.75-in. Pasteur pipet (Origio) in hyaluronidase solution (80 IU/mL; Sage In Vitro Fertilization/CooperSurgical). All four oocytes were zona-free, based on the observation under the inverted microscope at ×40 (Nikon Diapho/Hoffman). The other four oocytes (group 2) were dissected using a 1-mL syringe attached with 1.5-gauge needle, leaving one to three layers of corona cells intact; at this point the zona status was unconfirmed. The corona cells from group 2 were transferred to a culture medium where they separated from the oocytes, leaving all six fertilized zygotes zona–corona cell free. All six zygotes were cultured individually in two of four-well dishes (Nunc/Thermo Fisher Scientific) using Quinn's Advantage Protein Plus sequential media (CooperSurgical) under oil (Sage) for a total of 5 days.

The photographs shown in Figure 1 were taken at the time of the IVF procedure daily with imaging software (Zilos-tk Zona Infrared Laser Optical System; Hamilton Thorne Biosciences) on Nikon inverted microscope (Eclipse Ti; Nikon). The red circle in each of the photographs was the target indicator for the laser. The photographs were taken with a 35-mm focal length camera at horizontal and vertical resolutions of 96 dots per inch with 480–640 pixels.

RESULTS

Each of the eight oocytes successfully underwent ICSI, and six were fertilized equally among the groups, three from each group. The 11 oocytes retrieved were all zona-free, and eight oocytes were inseminated with ICSI. Out of the six fertilized zygotes, all cleaved on day 2; two embryos arrested at less than 30 cells, and one full blastocyst plus one late morula were available for embryo transfer (ET). Under abdominal ultrasound guidance approximately 2 cm from the fundus, transcervical ET was performed by one of the authors (M.T.) without difficulty using the Wallace SureView catheter (Fig. 1). Of the three zygotes in group 2, one failed to cleave on day 2, one arrested on day 3, and one arrested on day 5. Pregnancy was first detected with a serum quantitative human chorionic gonadotropin (hCG) level. The twins were delivered by scheduled cesarean delivery at an estimated gestational age of 37 weeks and 1 day.

DISCUSSION

Two reports (1, 2) describe four cases with oocytes demonstrating incomplete zona development and subsequent frozen-thaw embryo replacement. Stranger et al. (4) reported a pregnancy by ICSI of ZFOs. We present the first case of repetitive complete autologous ZFOs resulting in successful IVF-ICSI using fresh blastocyst transfer followed by the term delivery of healthy twins.

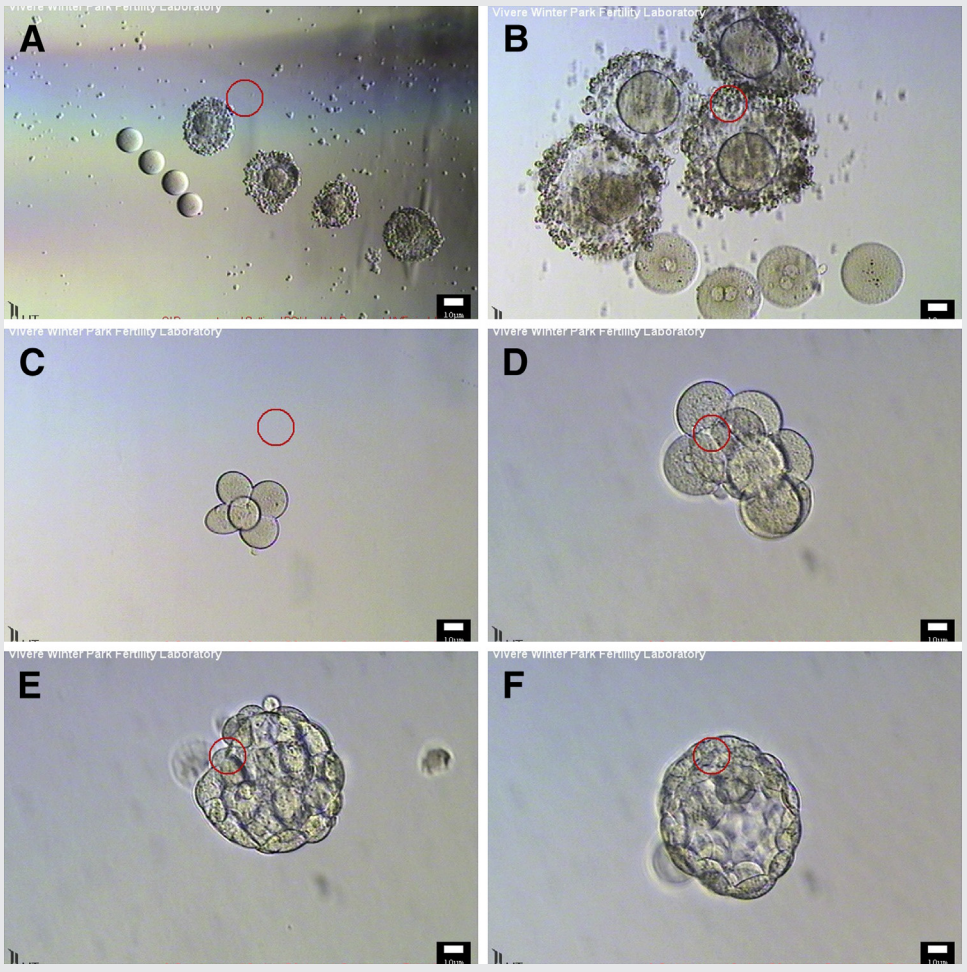
Usually ZFO occurs from abnormal zona production unless there is confirmed iatrogenic damage during oocyte aspiration or during cumulus removal for ICSI or fertilization assessment. During our patient's prior IVF cycle in another clinic, six oocytes with cumulus-corona cell intact were damaged during the process of cumulus removal. The 11 oocytes retrieved from our patient were all with cumulus-corona cell intact. Extreme care was taken to prevent any further damage during handling, given the patient's history of ZFO. To prevent any damage to the ZFOs, we used a nonpulled fire-polished Pasteur pipet rather than a stripper tip with a small opening. Four oocytes were successfully stripped without damage, and all ZFO underwent ICSI successfully.

To perform ICSI without damage, we attempted to leave some corona cells intact by using the technique that allows easier injection developed by Stranger et al. (4). Four oocytes were injected more easily with the corona cells intact. When transferring the zygotes to a new culture dish, we observed corona cells disengaging from the embryo, confirming all four oocytes were zona-free. Based on our observations, we believed the ZFO from this patient may have been due to abnormal zona production, given that no mechanical damage occurred during handling. We also recommend keeping corona cells intact to facilitate ICSI and replacing the media while maintaining the embryos in their original dish undisturbed.

Human oocytes are surrounded by corona and cumulus cells, collectively called cumulus packs, at the time of retrieval. Knowing this patient had a previous cycle with apparent ZFOs, we attempted to view the oocytes carefully under a Nikon inverted microscope. However, the presence of zona could not be confirmed. We used the spreading technique to confirm any presence of zona to appropriately avoid the stripping procedure with stripper tips. All 11 oocytes appeared zona-free, so four oocytes were stripped successfully without using any stripper tips. Further, unless ZFOs are suspected, the invasive and time-consuming spreading technique should be deferred.

For human IVF, fertilization is normally achieved by a conventional method or ICSI, depending on the sperm parameters. When encountering ZFO, fertilization should only be achieved by ICSI because the polyspermy block is

FIGURE 1



(**A**) Stripped and partially dissected oocytes before intracytoplasmic sperm injection (ICSI). (**B**) Day 1 after ICSI. (**C**) Day 2 after ICSI. (**D**) Day 3 after ICSI. (**E, F**) Day-5 embryos transferred.

Hu. Live birth from zona-free oocytes. Fertil Steril 2016.

at the level of zona pellucida. Ueno et al. (5) performed ICSI on all ZFO to avoid polyspermy, with the location of meiotic spindle confirmed beforehand to achieve good fertilization outcomes. The developmental potential of the ZFOs was similar to the zona intact oocytes in reference to fertilization, culture, and pregnancy outcomes (5). With the use of a time-lapse imaging system, Bodri et al. (6) also demonstrated that an oocyte with a removed, damaged zona could be successfully fertilized with ICSI, cultured until the blastocyst stage, and vitrified electively.

The blastomeres of zona-free embryos often coalesce during the cleavage stage when they are cultured in groups. To maintain individuality, the zone-free embryos must be cultured separately in a culture drop or well. Some blastomeres of zona-free embryos are easily disengaged or damaged during the cleavage stage from handling with a transfer pipette, so minimal manipulation of the embryos is mandatory. If changing the embryo culture medium is necessary, we recommend first removing approximately 90% of the existing medium, then adding pre-equilibrated fresh medium without disturbing the embryos. At the blastocyst stage, the embryos are more suitable for transfer or freezing compared with the cleavage stage.

Researchers have demonstrated beneficial effects on pregnancy outcomes in human IVF when transferring zona-free embryos at the blastocyst stage (3, 7–11). Lan et al. (8) demonstrated zona-free and laser zona-assisted hatching produced a comparative effect on blastocyst transfer in unselected IVF patients. Hiraoka, et al. (3) reported a successful pregnancy after vitrification of a human blastocyst that had completely escaped from the zona pellucida on day 6. Sampaio and Geber (10) reported births after transfer of zona-free blastocysts produced enzymatically. The live birth reported here may also demonstrate the positive effects on zona-free culture reported by other researchers. Patients with the rare condition of 100% zona-free oocytes maintain the potential for pregnancy after careful micromanipulation of the oocytes. Caution is recommended on the number of embryos selected for transfer to reduce the risk of multiple gestations.

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