Clinical pregnancy resulting from intracytoplasmic sperm injection of prematurely ovulated oocytes retrieved from the posterior cul-de-sac

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Objective: To report a clinical pregnancy resulting from intracytoplasmic sperm injection of prematurely ovulated oocytes retrieved from the posterior cul-de-sac.
Design: Case report.
Setting: Academic center.
Patient(s): A 40-year-old nulligravid woman underwent ovarian stimulation for in vitro fertilization (IVF). Daily injections of gonadotropin-releasing hormone antagonist were initiated on cycle day 8. A 10,000 IU dose of human chorionic gonadotropin was administered on cycle day 15 to trigger follicular maturation. The estradiol and luteinizing hormone levels on the trigger day were 1528 pg/mL and 2.4 mIU/mL, respectively. The patient underwent oocyte retrieval 35 hours after the trigger. Transvaginal sonography at the time of the retrieval revealed a large pocket of free fluid in the posterior cul-de-sac. Only 3 follicles measuring 10–12 mm were noted in both ovaries. No lead follicles were visualized.
Intervention(s): Aspiration of free fluid from the posterior cul-de-sac.
Main Outcome Measure(s): Clinical pregnancy.
Result(s): The fluid in the posterior cul-de-sac was aspirated, and 3 mature oocytes were retrieved. Aspiration of the smaller ovarian follicles measuring 10–12 mm did not yield oocytes. All mature oocytes retrieved from the posterior cul-de-sac were fertilized with intracytoplasmic sperm injection. Three cleavage-stage embryos were transferred 3 days later. A single intrauterine pregnancy with cardiac activity was confirmed at a gestational age of 7 weeks.
Conclusion(s): In the setting of premature ovulation, aspiration of free fluid from the posterior cul-de-sac can result in the retrieval of mature oocytes, which may result in clinical pregnancies. (Fertil Steril Rep® 2021;2:448–53. ©2021 by American Society for Reproductive Medicine.)
Key Words: Premature ovulation, premature LH surge, premature luteinization, gonadotropin-releasing hormone antagonist, ovarian stimulation, posterior cul-de-sac

INTRODUCTION

The follicular phase of the menstrual cycle is characterized by the selection of a dominant follicle and a corresponding rise in serum estradiol (E_2) level. This increase in E_2 stimulates a positive feedback loop that results in the preovulatory luteinizing hormone (LH) surge, which in turn is critical for oocyte maturation and ovulation. The administration of exogenous gonadotropins during ovarian stimulation results in the recruitment of multiple ovarian follicles, and therefore supraphysiologic serum E_2 levels. The early and accelerated rise of E_2 levels during ovarian stimulation can trigger a premature LH surge or premature luteinization, resulting in cycle cancelation or sub-optimal treatment outcomes (1, 2). Gonadotropin-releasing hormone (GnRH) antagonists and agonists

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are used routinely for the prevention of premature LH surge during ovarian stimulation (3). However, a breakthrough LH surge may occur in up to 4.3% of GnRH antagonist cycles (4) and 3% of GnRH agonist cycles (5). Although a breakthrough LH surge despite GnRH antagonist or GnRH agonist suppression is well documented in previously published literature, premature ovulation infrequently occurs during ovarian stimulation. There is also a dearth of information about the treatment outcomes of women with premature ovulation during ovarian stimulation. In this context, we report a clinical pregnancy resulting from intracytoplasmic sperm injection (ICSI) of prematurely ovulated oocytes during ovarian stimulation retrieved from the posterior cul-de-sac.

CASE REPORT

A 40-year-old nulligravid woman with a 2-year history of involuntary infertility presented to the office for evaluation. Her body mass index was 19.6 kg/m^2. Baseline ultrasonography revealed a normal uterus and an antral follicle count of 6. Her anti-müllerian hormone (AMH) level was 0.61 ng/mL. Hysterosalpingography demonstrated a normal uterine cavity and bilateral tubal patency. Her partner was a 47-year-old man with no pertinent urologic history. His semen analysis was normal, except for isolated teratozoospermia (strict morphology of 3%). The couple underwent 2 clomiphene citrate and intrauterine insemination cycles, but neither treatment cycle resulted in conception, because of which they underwent in vitro fertilization (IVF).

Given the patient's low ovarian reserve, an ovarian stimulation protocol consisting of oral clomiphene citrate and low-dose gonadotropins was chosen (6). Briefly, estrogen priming with 0.1 mg E_2 patches (Climara, Bayer Healthcare Pharmaceuticals, Berlin, Germany) was started in the preceding luteal phase. A 5-day course of 100 mg oral clomiphene citrate was started on cycle day 2; gonadotropin injections in the form of 150 units of Follistim (Merck, Kenilworth, NJ) and 75 units of Menopur (Ferring Pharmaceuticals Inc., Parsippany, NJ) was started on cycle day 5 (7). Suppression of a premature LH surge was achieved with the use of once-daily injections of ganirelix acetate (Merck, Kenilworth, NJ) beginning cycle day 8. The patient received the same dosage of gonadotropins and GnRH antagonist between cycle days 4 and 14. On cycle day 15, 6 follicles with sizes ranging between 13.7 to 23 mm were noted with serum E_2 and LH levels of 1528 pg/mL and 2.4 mIU/mL (Fig. 1). A 10,000 IU dose of human chorionic gonadotropin (hCG) was administered to trigger follicular maturation. The serum E_2 and β-hCG level on the day after the trigger was 1619 pg/mL and 95.8 mIU/mL, respectively. Table 1 summarizes the patient's ovarian stimulation protocol.

The patient underwent oocyte retrieval 35 hours after the hCG trigger. However, transvaginal sonography at the time of the retrieval revealed only 3 follicles measuring approximately 10–12 mm in both ovaries (Fig. 2A and B). Premature ovulation was suspected as the large follicles noted 2 days prior had collapsed, and a large pocket of free fluid was noted in the posterior cul-de-sac (Fig. 2C). The smaller ovarian follicles were aspirated under transvaginal ultrasound guidance, but no oocytes were retrieved. When the fluid in the posterior cul-de-sac was aspirated, 3 mature oocytes were retrieved. The retrieved oocytes were exposed to 40 IU recombinant hyaluronidase (Cumulase, Halozyme Therapeutics, Inc. San Diego, CA) to remove the cumulus cells (8). Intracytoplasmic sperm injection was performed using ejaculated sperm, and all the oocytes fertilized normally 14–17 hours after ICSI. All the embryos were cultured to the cleavage stage (Supplemental Video 1, available online). Three cleavage-stage embryos with grades 2.5, 3, and 3.5 (Fig. 3) were transferred using a Wallace catheter (Smiths Medical Inc., Norwell, MA). The β-hCG levels 11, 13 and 20 days after the embryo transfer were 183 mIU/ml, 414 mIU/mL and 3,933 mIU/mL, respectively. A single intrauterine gestational sac with a yolk sac was seen on cycle day 37. A single intrauterine pregnancy with cardiac activity was noted at a gestational age of 7 weeks (Fig. 3A and B). The patient experienced a spontaneous miscarriage at 9 weeks, though her obstetrician did not test the products of conception. However, we posit that the miscarriage occurred because of age-related aneuploidy. The patient underwent 2 subsequent IVF cycles with double the dose of GnRH antagonist i.e., daily dosing in the morning and evening. Premature LH surge or ovulation did not occur in either cycle. Both cycles yielded 2–3 mature oocytes, and 1–2 cleavage-stage embryos were transferred on day 3. Neither cycle resulted in conception.

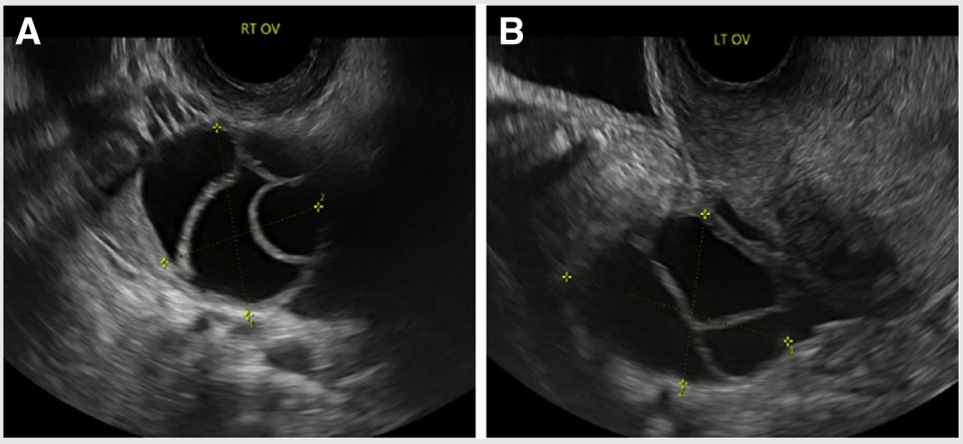
Institutional review board approval was not required for this case report as per our institution's policy. Patient consent was obtained for the publication of the case.

DISCUSSION

Premature ovulation during IVF is rare, with an estimated incidence of 0.34%, and is more commonly reported closer to oocyte retrieval (8–10). However, when it does occur, premature ovulation often results in IVF cycle cancelation, retrieval of no oocytes, or poor quality oocytes that fail to fertilize (8, 10). Patients with a diminished ovarian reserve are thought to be at a higher risk for premature ovulation even in the setting of adequate GnRH antagonist down-regulation (9).

The management of a patient with premature ovulation before oocyte retrieval is challenging. Anecdotally, oocytes from the remaining smaller follicles are thought to have a lower fertilization potential, presumably due to immaturity (8, 10, 11). However, in one case series of 3 patients, Wu et al. (10) demonstrated successful pregnancy outcomes after documented premature ovulation during GnRH antagonist-based IVF cycles. The first patient was a 28-year-old G0 woman with polycystic ovarian syndrome where the E_2, LH, and progesterone levels on the day of trigger (cycle day 16) was 855.9 pg/mL, 0.5 mIU/mL, and 0.96 ng/mL, respectively. Transvaginal oocyte retrieval was performed 36 hours later; however, none of the larger lead follicles were visualized. Six metaphase I (MI) oocytes were retrieved from medium-sized follicles measuring 12–14 mm. Four oocytes reached the metaphase II (MII) stage the next day and were fertilized with ICSI. Four cleavage-stage embryos were transferred 3 days later, and a live female infant weighing 2,452 g was

FIGURE 1



Transvaginal ultrasonography on the day of human chorionic gonadotropin trigger shows 6 follicles with sizes ranging between 13.7 and 23 mm in the right ovary (**A**) and left ovary (**B**).

Pereira. Posterior cul-de-sac oocyte retrieval. Fertil Steril Rep 2021.

delivered at 36 weeks. The second patient was a 34-year-old G0 woman with bilateral tubal occlusion, who had 3 lead follicles with the sizes of 20 mm, 19 mm, and 17 mm on cycle day 12. The E_2, LH, and progesterone levels on the day of trigger were 1,125 pg/mL, 0.64 mIU/mL, and 1.6 ng/mL, respectively. No lead follicles were visualized on the day of the retrieval. However, a large amount of free fluid was noted in the posterior cul-de-sac. Six MI oocytes were retrieved from the smaller follicles transvaginally; 4 matured overnight. Only 1 MII oocyte was fertilized, which was transferred as a cleavage-stage embryo 3 days later. A live male infant weighing 4,006 g was delivered at 37 weeks. The last patient was a 40-year-old woman with endometriosis and 3 prior failed IVF cycles. The trigger was administered on cycle day 13, and the corresponding E_2 and progesterone levels were 1,198.8 pg/mL and 1.9 ng/mL, respectively. No lead follicles were seen on the day of the retrieval, but free fluid was noted in the posterior cul-de-sac. A postmature oocyte was noted in the aspirated fluid from the posterior cul-de-sac transvaginally. Four MII oocytes were retrieved from the medium and small-sized follicles. Two oocytes were fertilized with ICSI, and 2 cleavage-stage embryos were transferred 3 days later. A live male infant weighing 3264 g was delivered at 38 weeks.

Consistent with the above case series, our patient experienced premature ovulation before oocyte retrieval, despite having no imminent signs of a premature LH surge or spontaneous ovulation on the day of or after the trigger. Like the above case series, our patient underwent ovarian stimulation with a GnRH antagonist ovarian stimulation protocol. In contrast, all oocytes in the current patient were retrieved by aspirating the free fluid in the posterior cul-de-sac via transvaginal sonographic guidance, while the smaller size follicles did not yield any oocytes.

It is important to distinguish premature ovulation from a premature LH surge during ovarian stimulation. The latter is defined as an LH level of $\geq$10 mIU/mL, and a progesterone level of $\geq$1.0 ng/mL before the criteria for hCG trigger is met (5, 11). While completion of IVF cycles and retrieval of oocytes is still possible with a premature LH surge (12, 13), the rise in progesterone levels usually results in endometrial asynchrony, thereby necessitating cryopreservation of embryos (13). As exemplified by our case and the case series by Wu et al. (10), premature ovulation may occur without imminent signs of an LH surge and presents most often at the time of the oocyte retrieval. The fertilization potential of prematurely ovulated oocytes remains unknown. However, a study of 93 IVF cycles with laparoscopic retrievals by Dirnfield et al. (14) reported similar fertilization and cleavage rates of oocytes retrieved from the posterior cul-de-sac when compared with oocytes retrieved directly from ovarian follicles. The investigators also noted a significant decrease in the fertilization rate of oocytes that were aspirated from the posterior cul-de-sac after 60 minutes of beginning the laparoscopic retrieval. In another study, Matson et al. (15) compared the fertilization rate of spontaneously ovulated oocytes and oocytes retrieved from intact ovarian follicles. The investigators noted no differences in the fertilization rates of the 2 groups. One woman with spontaneous ovulation had a live birth after the transfer of 4 cleavage-stage embryos that were generated from the fertilization of oocytes aspirated laparoscopically from the posterior cul-de-sac. Given that laparoscopic oocyte retrieval was considered the standard of care in the mid-to-late 1980s, patients in the Dirnfield et al. (14) and Matson et al. (15) studies underwent laparoscopic oocyte retrievals.

To summarize, the current case highlights that premature ovulation may occur in IVF cycles despite GnRH antagonist down-regulation, although this is reassuringly rare (9). Physicians may opt to cancel such IVF cycles because of the risk of retrieving no oocytes or retrieving oocytes with poor fertilization potential from the remaining small and medium-sized follicles (10). Physicians may also choose to perform

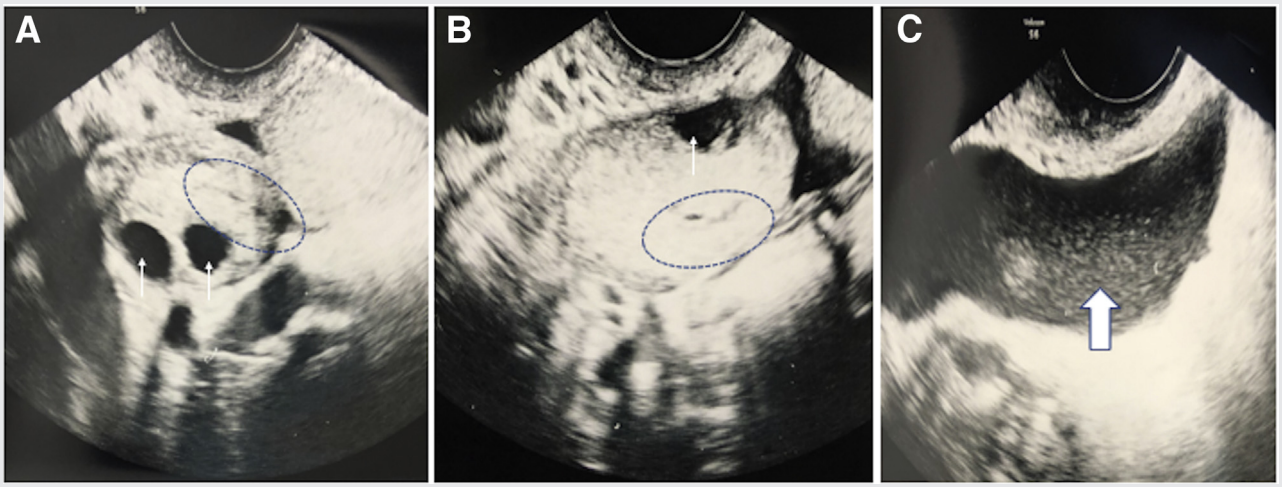
TABLE 1

Summary of the patient's ovarian stimulation protocol.

Cycle day	Day of cycle start	Cycle day 7	Cycle day 9	Cycle day 11	Cycle day 12	Cycle day 13	Cycle day 14	Cycle day 15
Estradiol (pg/mL)	38.9	158	302	588	713	877	1109	1528
Follicle-stimulating hormone (mIU/mL)	4.4	-	-	-	-	-	-	-
Luteinizing hormone (mIU/mL)	2.6	1.4	1.6	1.2	1.4	1.5	1.7	2.4
Lead follicular size (right ovary)	4<10 mm	11 mm, 7.4 mm, 7.1 mm	14.5 mm, 11.5 mm, 7.3 mm	18.1 mm, 15.8 mm, 7.7 mm	18.5 mm, 173 mm, 8.5 mm	19.4 mm, 18.4 mm, 11.5 mm	19.5 mm, 19.5 mm, 11.5 mm	23 mm, 21.5 mm, 13.7 mm
Lead follicular size (left ovary)	4<10 mm	8.8 mm, 8.2 mm	13.6 mm, 12.5 mm	15 mm, 14.5 mm, 8.5 mm	17.5 mm, 15.5 mm, 11.5 mm	17.8 mm, 16.3 mm, 12.4 mm	18 mm, 16 mm, 11 mm	20.5 mm, 19.5 mm, 14.5 mm
Endometrial stripe thickness (mm)	5.9	4.7	4.5	7.7	8.5	7.8	9	9.2

Pereira. Posterior cul-de-sac oocyte retrieval. Fertil Steril Rep 2021.

FIGURE 2



Transvaginal ultrasonography on the day of oocyte retrieval showing collapsed follicles (*blue dotted oval*) in the right ovary (**A**) and left ovary (**B**). Smaller follicles measuring approximately 10–12 mm are noted in both ovaries (*white arrows*, **A** and **B)**. A large pocket of free fluid (*solid white arrow*) was noted in the posterior cul-de-sac (**C**). These findings were suspicious for premature ovulation.

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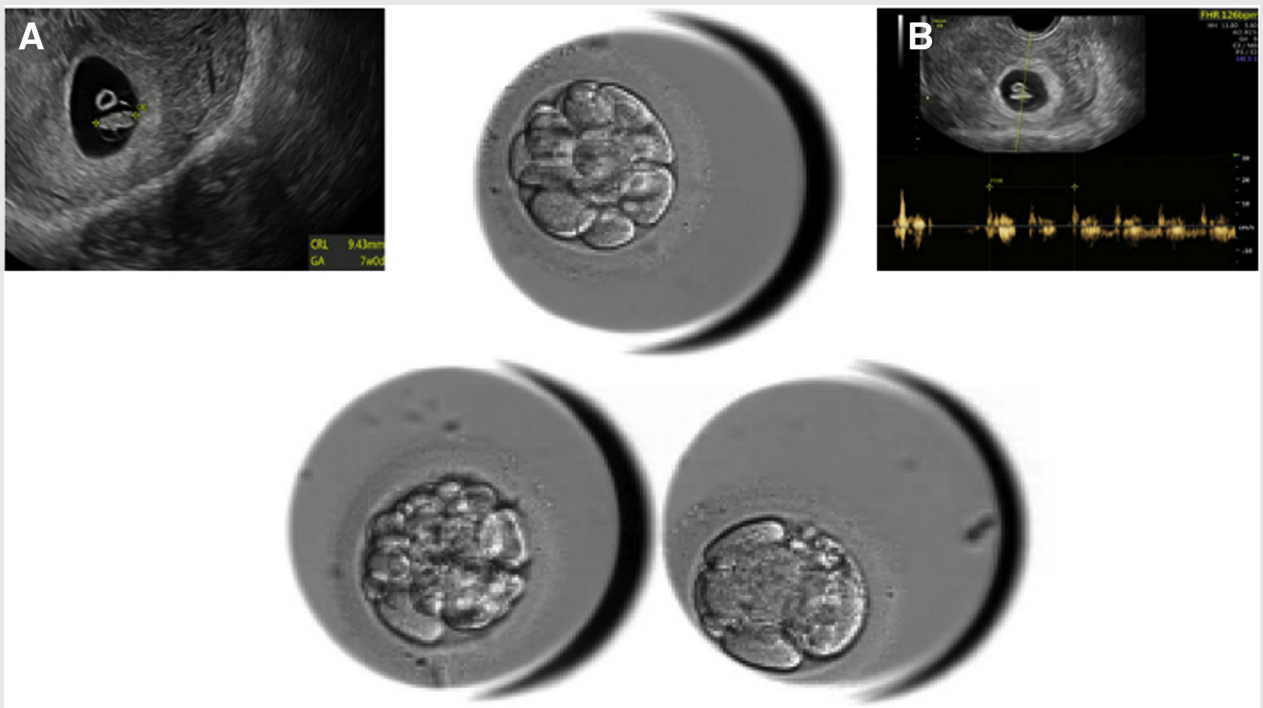
FIGURE 3



Three cleavage-stage embryos (7-cell, 6-cell, and 5-cell) were transferred. A single intrauterine pregnancy (*inset*, **A**) was noted at a gestational age of 7 weeks with the fetal cardiac activity of 126/min (*inset*, **B**).

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intrauterine insemination instead of oocyte retrieval, especially in patients with tubal patency. However, in the setting of premature ovulation, aspiration of free fluid from the posterior cul-de-sac via transvaginal sonographic guidance can increase the chance of retrieving mature oocytes, which can retain normal fertilization capacity and embryo

development, occasionally resulting in clinical pregnancies or live births (10, 15).

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