Natural cycle IVF/IVM may be more desirable for poor responder patients after failure of stimulated cycles

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Received: 15 March 2011 / Accepted: 31 May 2011 / Published online: 22 June 2011
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Abstract

Objective To describe pregnancies and live births resulted from natural cycle IVF combined with in-vitro maturation (natural-cycle IVF/IVM) for three poor responder women after failure of stimulated cycles.

Methods For case 1 and 2, the mature oocytes from aspirated follicles were inseminated by intracytoplasmic sperm injection (ICSI) immediately; immature oocytes were matured in vitro, and the embryos from mature and immature oocytes transferred on day 3 after oocyte retrieval. For case 3, 3 consecutive natural cycles were performed, in which the matured oocytes from in vivo or in vitro were vitrified in the first and second cycle, and warmed on the retrieval day of the third fresh cycle. Then the embryos resulted from vitrified-warmed and fresh oocytes were transferred.

Results A total of 15 oocytes were obtained from the 7 retrieval cycles for the three cases. The case 1 was successfully pregnant at her first natural cycle, and case 2 was pregnant after two consecutive cycles. The two cases had successfully delivered and case 3 was in her ongoing pregnancy at the time of submission.

Conclusion These results demonstrate that natural-cycle IVF/IVM might be a reasonable and efficient treatment alternative for poor responder patients when stimulated cycle has failed.

Keywords Natural cycle · In vitro fertilization · In vitro maturation · Pregnancy · Poor responder

Introduction

The natural cycle in vitro fertilization (IVF) produced the first IVF baby in the world. But it was soon replaced by the controlled ovarian hyperstimulation (COH) cycles. However, ovarian stimulation is not free from negative consequences and risks, including ovarian hyperstimulation syndrome. These potential problems are even more evident in poor-responder patients, where only very few follicles can be recruited and very few oocytes can be retrieved after stimulation despite the high dose of gonadotropins administered or repeated stimulated cycles performed and their management remains a challenge in assisted reproduction. For these patients with poor responding, if repeated stimulated IVF cycles are unsuccessful, patients especially of advanced age are considered to have a poor clinical outcome compared with both young and those with normal response [1].

With the increasing awareness of side effects of ovarian stimulation and better understanding of ovarian physiology in relation to ovarian follicular growth and maturation, IVF in natural cycles has gained great attention and interest for both normal responder and poor responder patients [2–6] because natural cycle offers a more physiological, less drug-oriented, lower risk and more patient-friendly approach. However, the efficacy of natural IVF is hampered by the high incidence of oocyte retrieval failure (16.7–71.4%) and the relatively low pregnancy rate per embryo transfer (ET) cycle (0–23.5%) [2, 4, 7–11]. And only effective treatment with 29.2% pregnancy per transfer by natural-cycle IVF was observed in 35 years old or younger

Capsule Natural-cycle in vitro fertilization combined with in vitro maturation of immature oocytes might be an efficient treatment alternative for poor responder patients when stimulated cycle has failed

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poor responder patients by Schimberni et al. (9). However, in these previous natural IVF cycles, generally, only the dominant follicle was aspirated while the other few small antral follicles were not [9]. Also, the immature oocyte retrieved from the small follicles followed by IVM has also been a successful treatment for infertile women with polycystic ovary syndrome (PCOS), and could get approximately 30% pregnancy rate [12]. Furthermore, the combination of natural cycle IVF with IVM of immature oocytes (natural cycle IVF/IVM) has successfully resulted in pregnancy for infertility and has been looked upon with an increasing interest as a novel treatment [13, 14]. Moreover, natural cycle IVF/IVM has shown to be efficient for infertility treatment of various causes with acceptable pregnancy and live birth rates in our recent study [15].

Thus, if the strategy of natural IVF/IVM cycle is feasible for women with poor responses, it may be especially precious for the poor responder women because the few oocytes from dominant and small follicles could be effectively and optimally used. However, the real place for it has yet to be defined as we lack published data.

This case report presents the primary evidence of three successful pregnancies via natural IVF/IVM cycles treatment for poor responders when stimulated IVF cycles had failed.

Materials and methods

The study was approved by the Hospital Institutional Review Board. The natural cycle IVF combined with IVM treatment was explained to the patients, and the written informed consent was obtained. A total of 30 retrieval cycles were performed for poor responder patients by natural-cycle IVF/IVM and 11 patients got pregnant (36.7%). Out of them, 15 poor responding patients after failure of stimulated cycles were enrolled in this natural cycle program. A total of 20 oocyte pickup cycles was performed for them and 17 ET cycles was completed, which resulted in 5 pregnancy and 2 out of them (both aged at 40 years) were miscarried. The pregnancy rate was 25.0%, 29.4% and 33.3% per OPU, per ET and per patient, respectively for these poor responders with failure of stimulated cycles. The details of the three successful pregnancies and subsequent live birth for poor responder women with stimulated cycles failure are described below.

Case reports

Case 1

A 34-year-old woman with a regular menstrual cycle with 25- to 30-day intervals was infertile because of bilateral

tubal obstructions. Previous two stimulated cycles include a long protocol and a short protocol with only one oocyte obtained each cycle and no ET was performed in the second cycle. After 2 years, the patient visited our center and wanted the natural cycle IVF/IVM treatment because of the repeated failures by stimulated IVF cycles.

On the day 15 of this cycle, a leading follicle with the size of 16 mm in diameter and 2–3 small follicles in her right ovary were revealed by the ultrasound scan. On day 10 of the following menstrual cycle, one follicle was revealed in the left ovary and 2–3 follicles in the right. When the dominant follicle reached 12.5 mm in diameter on day 12, human chorionic gonadotropin (hCG) was administered (10,000 IU). The oocyte retrieval was performed 36 h later as we previously described [15]. No oocyte was obtained from the leading follicle as the oocyte had ovulated and 3 oocytes were collected from the small follicles with one metaphase II (MII) and two germinal vesicle (GV). The mature oocyte was inseminated 3 h later by intracytoplasmic sperm injection (ICSI). The immature oocytes were matured after 24 h in IVM medium containing 30% of the patient's own serum (inactivated at 56°C for 30 min) supplemented with FSH (75 mIU/mL), 10 mIU/ml human menopausal gonadotrophin (Livzon Medical Groups; Zhu Hai, China) and 10 ng/ml recombinant human epidermal growth factor (Invitrogen, Carlsbad, CA, USA) and were inseminated by ICSI. All of the 3 oocytes from small follicles were fertilized and cleaved.

The endometrial preparation and luteal support were also operated as we previously described [15]. On day 3 after oocyte retrieval, the embryos from mature and immature oocytes were transferred together. The embryos produced from mature oocytes are on day 3 and embryos produced from immature oocytes are on day 2 [14]. If there were no mature oocytes on the day of collection, the embryos were transferred on day 4. The embryos derived from the mature and in vitro matured oocytes were all at 4-cell stage for this case. On day 14 following ET, the level of serum β -hCG was 213 mIU/ml, and at day 27 after ET, an intrauterine singleton pregnancy with a fetal heartbeat was observed via transvaginal ultrasonography. At 5 weeks after ET, the fetus was in good condition, and a 3,600 g healthy girl was delivered at 39 weeks' gestation.

Case 2

A 35-year-old woman with a regular, 24-day-interval menstrual cycle had salpingectomy of the right side after ectopic pregnancy followed by tubal obstructions on the left. One previous stimulated cycle was performed but given up halfway because of the poor responding and asynchrony development of follicles during the stimulation. Three months later, the patient came to our clinic for the

natural cycle treatment. On day 4 of her menstrual cycle, 2–3 small follicles in each side were revealed with a follicle at 10.7 mm in diameter in her right ovary. On day 8, the dominant follicle reached 14.5 mm in diameter, hCG was administered. After 36 h later, one oocyte at metaphase-I (MI) stage was retrieved from the dominant follicle and three oocytes (two at MII stage and one at GV stage) were retrieved from small follicles. The MI stage oocyte derived from the leading follicle was seen as abnormal morphology after 24 h maturation in culture and the other oocyte from the small follicle was still at GV stage 48 h later then discarded. On day 3 after oocyte retrieval, two embryos derived from small follicles were transferred: one was at the 8-cell stage and the other was at 4-cell stage. 14 days later after ET, no pregnancy occurred.

On day 2 of her next menses, 5–6 follicles were seen in both ovaries. On day 9 of the cycle, the dominant follicle was 12.5 mm in diameter and hCG was administered. Two mature oocytes (one from the dominant follicle and the other from small follicles) were collected at the time of retrieval. The two resulted embryos were transferred on day 3: the embryo derived from the leading follicle was at the 5-cell stage and the other from the small follicle was at 7-cell stage. On 14 days after ET, the serum β -hCG level was 677 mIU/ml. At 5 weeks after ET, two gestational sac were detected on ultrasound scan (one was detected with a fetal heartbeat but the other not). 2 months after ET, the fetus with heartbeat was going well while the other was seen as a small gestational sac (0.8 cm). At 38 weeks' gestation, a 3,500 g healthy boy was delivered.

Case 3

A 41-year-old woman with a regular menstrual cycle had been infertile for 16 years. Her husband was diagnosed as azoospermia. They experienced the failure of a stimulated and a minimal stimulation cycle with fresh sperm retrieved by percutaneous epididymal sperm aspiration (PESA) in our clinic. Considering the reduced numbers of follicles and the repetitive PESA, the couples insisted on and decided the natural cycle treatment and agreed to cryopreserve the oocytes for two consecutive natural cycles. Then in the next two menses, the collected oocytes were all frozen at MII stage by vitrification. On day 2 of the first natural cycle, the serum FSH level was 14.3 mIU/ml. On day 9 of her cycle, a dominant follicle was revealed at 13.4 mm in diameter and hCG was administered. Two oocytes were retrieved (a mature oocyte from dominant follicle and a GV oocyte from small follicle). The mature oocyte was vitrified 2 h later after retrieval. The GV oocyte reached MII stage after 24 h maturation in culture and was vitrified. In the second retrieving-freezing cycle, only one oocyte at MII stage was retrieved from the dominant

follicle and was cryopreserved. On day 4 of the third successive menses, the serum FSH level was 19.4 mIU/ml. Two mature oocytes were retrieved (one from the dominant follicle and the other from the small follicles) on day 12 of this cycle. And the 3 vitrified oocytes in the previous two cycles were warmed on the retrieval day of the third cycle: two oocytes frozen in the first cycle were both degenerated during the warming and the oocyte frozen in the second cycle was survived. The warmed-survived and two fresh oocytes were all inseminated by ICSI using sperm by PESA and fertilized. The embryos from fresh oocytes were at 8-cell and 7-cell stage, respectively and the embryo from frozen-warmed oocyte was at 3-cell stage on day 3. They were all transferred on day 3. On 14 days following ET, the serum β -hCG level 896 mIU/ml and an intrauterine singleton pregnancy with a fetal heartbeat was observed via transvaginal ultrasound scan at day 28 after ET. Now the patient had a 31 week's ongoing pregnancy at the time of submission.

Discussion

In the current case report, we present three pregnancies in poor responders by the treatment of natural cycle in vitro fertilization combined with in vitro maturation of immature oocytes and demonstrates that natural cycle IVF/IVM treatment could be an efficient treatment alternative for the poor responder women when stimulated cycles has failed or cancelled.

Poor response to gonadotropin stimulation occurs more often in older women, but may also present in young women [16, 17]. The majority of poor responder women had advanced reproductive age, diminished antral follicle count, elevated day-3 FSH concentrations or previous failed cycles. In the context of a poor responder woman, natural cycle could yield better quality oocyte by natural follicle selection and allow transfer on endometrium whose receptivity has not been distorted by stimulation [18]. In our clinic, natural cycles were undertaken as a last resort treatment for them before abandon fertility treatment. The above unfavorable prognostic characteristics could also be revealed for the present three poor responders and pregnancies were successfully achieved via natural cycle IVF/IVM for them.

As seen from case 1 and 3, the immature oocytes from small follicles could be matured in vitro after the selection or even ovulation of the leading follicle has occurred at the time of retrieval. These further confirmed that the developmental competence of oocytes from small follicles is not affected either by the presence of a dominant follicle or by the phase of folliculogenesis [19]. Also, in small follicles, mature oocytes could be obtained at the time of retrieval in case 2, which is consistent with our recent results [15].

From the current study, each of these oocytes from leading or small follicles of the remaining few follicles seems to have the same chance to be matured in vitro and to achieve pregnancy in poor responders. This suggests for the physicians to do all they can to obtain the oocytes from the existing follicles even only one follicle recruited. Because the presence of an extra embryo available for transfer decreases the risk of cycle cancellation and changes the chance of pregnancy substantially. Moreover, an acceptable pregnant rate (35.9%) was obtained in our recent natural cycle IVF/IVM treatment [15]. And the pregnant rate per cycle (36.7%) for poor responder patients by natural cycle IVF/IVM in our center was higher than that of our not published results (15.4%) and reported [2, 4, 7–11] by natural IVF cycle. These results further demonstrate that natural cycle IVF/IVM is an efficient technology and could be a desirable and feasible alternative for poor responder patients who has very low number of follicles and for whom the stimulation could not work.

In case 3, the infertility was mixed with poor responding and azoospermia. Moreover, the case 3 shared all the poor characteristics of poor responders: advanced reproductive age, fewer follicles recruited, higher basal FSH concentrations compared with the other two cases. The previous attempt of minimal stimulation for her did not seem to have advantage over natural cycles in terms of oocyte number and pregnancy improvement with additional costs as well as stress. This is consistent with the results of Schimberni et al. (2009) [9] and an earlier study that minimal stimulation IVF is not an effective treatment for poor responders with high FSH levels and low numbers of oocytes retrieved in a previous IVF cycle [20]. And the retrieved oocytes matured in vivo or in vitro were vitrified for two consecutive cycles by natural cycle to avoid the repeated PESA. Though only one out of three vitrified oocytes was survived after warming and transferred together with the fresh ones, to our delight, the pregnancy gives us a new way to attempt for this kind of patients. Also, 3 consecutive natural cycles were performed and preferred by the couple because of the easier procedure, lower cost and less physical and emotional stress for both the wife and her husband.

Furthermore, at least four consecutive attempts were suggested in the counseling of the poor responders because the chance of oocyte recovery and pregnancy per cycle remains the same, independent of whether they were the first, second, third, fourth, or further consecutive cycle by natural IVF cycle [9]. Moreover, cumulative pregnancy rates are more relevant than pregnancy rates per started cycle to evaluate effectiveness of natural cycle because of the monthly, patient-friendly and easy repeatability. It has been reported that cumulative ongoing pregnancy rate was 34.0% after three cycles of minimal stimulation IVF [21], 43% and 42% after three and five natural oocyte pickup

cycles [22, 23] and 46% after four started natural cycles [3]. Previous studies also found that the natural cycle works at least as well as the COH in poor responder women who failed previous ovarian hyperstimulation [9, 24]. In the present case report, the case 1 was successfully pregnant at her first natural IVF/IVM cycle. The other two cases got pregnant after at least two consecutive natural cycles. Therefore, the cumulative pregnancy rate is also done for natural IVF/IVM cycle. These further suggest for the physicians that consecutive natural IVF/IVM cycles should be discussed as an option for such women with poor prognostic characteristics before offered oocyte donation or proceed to abandon fertility treatment.

In conclusion, natural cycle IVF/IVM treatment for poor responders may be a reasonable and effective treatment alternative to ovarian stimulation because of its easy, monthly repeatability and because of the more oocytes retrieved compared with natural cycle IVF. This treatment maximizes the treatment efficacy by combining the advantages of natural cycle IVF and IVM, especially desirable for women with poor-responding who has only very few follicles recruited and very few oocytes retrieved after stimulated cycles. However, to assess the real efficacy and to establish the role of this approach in poor responder patients, prospective randomized trials of adequate size are needed to guide these women appropriately.

Acknowledgements We are grateful for all the clinical, nursing, embryology, and administrative staff of the reproduction team for their support.

Conflict of interest Neither I nor my spouse/partner has any conflict of interest to declare.

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