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A case report of clinical pregnancy in which oocyte retrieval was carried out in the early follicular phase of the menstrual cycle

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Summary

To report a clinical pregnancy after frozen embryo transfer (FET) for which oocyte was picked up in the early follicular phase of the menstrual cycle. A 40-year-old woman with a two-year history of primary infertility was recruited. After in vitro fertilization (IVF), the embryo underwent vitrification cryopreservation process and FET was carried out five months later. The patient successfully conceived after FET. Follicle recruitment and selection cannot only be performed during a female patient's interovulatory interval (IOI), but oocytes can also be picked up in the early follicular phase of the menstrual cycle conception through IVF, embryo vitrification cryopreservation, and FET. Oocyte retrieval in the early follicular phase of the menstrual cycle increases the chances of an infertile female to conceive, especially in cancer patients and in women with poor ovarian response (POR).

Key words: Early follicular phase; In vitro fertilization; Poor ovarian response.

Introduction

In the past few decades, experiments have been conducted on animals as well as on humans to explore the dynamic growth and endocrine function of ovarian follicles. Accompanied by the development of embryology, measures like in vitro fertilization-embryo transfer (IVF-ET), embryo cryopreservation, and thawing, can be implemented to solve the problem of infertility. Nevertheless, the case report the present authors present shows several aspects that are still not fully understood. They describe growth features, endocrine condition, and developmental potential of mature follicle found in the early follicular phase, and they also discuss the practical significance of oocyte retrieval in the early follicular phase.

Case Report

A 40-year-old woman with a two-year history of primary infertility was examined at the Reproductive Medicine Center of Tianjin Central Hospital of Obstetrics and Gynecology. The diagnosis of her infertility was poor ovarian response (POR) and fallopian tube disease. Poor ovarian response was defined in accordance with the Bologna criteria [1]. At least two of the following three features must be present to establish the definition: (i) advanced maternal age (≥ 40 years) or any other risk factor for POR, (ii) a previous POR (≤ 3 oocytes with a conventional stimulation protocol), and (iii) an abnormal ovarian reserve test (i.e. AFC, 5–7 follicles or AMH, 0.5–1.1 ng/ml). Her basic endocrine screening was shown as follows: follicle-stimulating hormone (FSH): 47.4 mIU/ml, luteinizing hormone (LH): 16.8 mIU/m, thyroid-stimulating hormone (TSH): 1.493 uIU/ml, prolactin: 5.15 ng/ml, estradiol (E_2): 50.2 pg/ml, and testosterone (T): 15.9 ng/dl. High levels of FSH, LH as well as E_2 indicated her poor ovarian reserve. However, the patient was able to maintain a regular menstrual cycle. After one-year treatment using traditional Chinese medicine with unsatisfactory outcomes, she and her husband decided to undergo IVF.

The most recent test report of her husband's semen analysis showed a normal volume of 5 ml, sperm concentration of 25 million/ml, and 48% motility with forward progression. The sperm parameters after processing using swim-up method were 27 million sperm with 90% progressive motility on the day of IVF. On day 2 of the menstrual cycle, vaginal ultrasound scan showed two follicles attaining a diameter of 13 mm, with the following endocrine level: FSH 5.50 mIU/ml, E_2: 149.9 pg/ml, and LH: 4.43 mIU/ml. Then 225 units of human menopausal gonadotropin (hMG) was given from day 2 to 3. On day 4, the follicle diameters reached 15 mm and 15.5 mm, while thickness of endometrium was 5.7 mm; E_2 level increased to 254.83 pg/ml and LH level increased to 12.02 mIU/ml. The stimulation protocol ended on cycle day 4 with the administration of 5,000 IU of human chorionic gonadotropin (hCG), and one oocyte was acquired 36 hours later (Table 1).

Fertilization resulted from conventional IVF. Eighteen hours after insemination and removal of cumulus cells, only one oocyte was fertilized with two pronuclei. Forty-eight hours after oocyte retrieval, the embryo was at the stage of four-cell grade 1, where grade 1 represents the highest quality. Considering the endometrium might be out of phase, the authors advised the patient and her husband to cryopreserve the embryo and undergo FET in a subsequent cycle. The couple agreed to this advice.

After three menstrual cycles, the patient returned to thaw the embryo. Starting on day 2 of the cycle, 3-5 mg estradiol was administered once or twice a day. The present authors found that the

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Table 1. — *The measurements of follicles size, hormone level , and endometrial thickness during the described stimulation cycle, as well as the medications used during this treatment cycle.*

Treatment cycle	1 day	2 day	3 day	4 day
hMG (unit)		225	225	
hCG (IU)				5000
Monitoring				
FSH (mIU/ml)		5.5		
LH (mIU/ml)		4.43		12.02
E_2 (pg/ml)		149.9		254.83
Follicles size (mm)		13+13		15.5+15
Endometrial thickness (mm)				5.7

hMG: human menopausal gonadotropin, hCG: human chorionic gonadotropin, FSH: follicle-stimulating hormone, LH: luteinizing hormone, E2: estradiol.

thickness of endometrium was 11.4 mm on day 28 and level of E_2 achieved was 985 pg/ml while level of progesterone was 0.3 ng/ml. From then on, 30-60 mg progesterone injection were added until day 32 when vaginal ultrasound scan showed the endometrium with a thickness of 10.9 mm and level of E_2 was 877 pg/ml while progesterone level increased to 10.9 ng/ml. On cycle day 33, the frozen embryo was thawed to transfer to the uterus of the patient. Luteal management included the administration of 4 mg estradiol twice daily and 20 mg progesterone injection five times daily from day of FET until 14 days after transfer. A serum β-hCG level of 258 IU/L was detected 15 days after embryo transfer which increased to 2,789 IU/L after 20 days of FET. A vaginal ultrasound performed four weeks later confirmed the presence of a single intrauterine gestational sac containing a viable fetus.

Discussion

This report suggests that we should re-recognize the process of follicle recruitment and selection. There are three major theories in regards to follicle recruitment. The first one is continuous recruitment. Animal experiments suggest that follicle recruitment is a continuous process [2]. According to this theory, antral follicles of diameter ≤ 4-6 mm can grow continuously in women of child-bearing age independent of gonadotropins[3]. The follicle which can ovulate is selected from continuous mature follicles by chance and accomplishes ovulation by the rise of LH. The second theory is single recruitment. In this theory, it is thought that the recruitment occurs among antral follicles with diameters of 2-5 mm at comparison, but not with identical stages of development. The follicle ovulated in the final is selected, by chance, from the pool following luteal regression [4]. Nowadays, more and more researchers approve the third theory of follicle recruitment called waves recruitment. Waves refer to a group of antral follicles develop synchronously at regular intervals during the menstrual cycle. Follicles in each wave are similar but not identical in diameter [5]. The earliest research on follicular dynamics found that the recruitment occurred twice during a menstrual cycle: the first recruitment occurred in the follicular phase while the second recruitment occurred in the luteal phase [6]. Evidence of follicular waves has been reported in non-human primates [7]. Furthermore, Baerwald *et al.* studied the follicle recruitment of 50 females of child-bearing age with regular menstrual cycles. They found that 34 women (68%) exhibited two waves of follicle recruitment during the IOI, while the remaining 16 women (32%) exhibited three waves [8]. Follicle selection refers to the process by which a single dominant follicle is chosen from the recruited waves for preferential growth. Previously, it was widely believed that the selection process occurs only once during IOI and it generally occurs in the early- to mid-follicular phase of the menstrual cycle when the dominant follicle achieves 10 mm diameter [9]. In contrast to this opinion, Baerwald *et al.* described the selection process appearing more than once in most healthy women and that it is usually associated with follicle recruitment [6]. Follicle recruitment occurs in the early follicular phase of the menstrual cycle and the dominant follicle is selected for ovulation in most females. However there is always another one or two recruitments of follicles in the preceding luteal phase where dominancy is not manifested and the selected follicle will not ovulate [6]. In this case report, the authors picked the oocyte on cycle day 6, in other words, in the early follicular phase of the menstrual cycle. This may suggest that at least one recruitment occurs in the preceding luteal phase and the selection process occurs later on. Whether the dominant follicle would have ovulated itself or regressed was unclear because the authors obtained the oocyte by the method of artificial retrieval. It has been reported that follicles picked in the luteal phase could be fertilized normally by means of in vitro maturation (IVM) and develop to normal embryos[10], so antral follicles in luteal phase may not be atretic, as they are likely to be in an early follicular phase of the menstrual cycle. The existence of healthy antral follicles proves that the recruitment of follicle in luteal phase is possible. In this case report, the follicle picked up was also fertilized and developed to four-cell grade 1, which demonstrates the high quality of the follicle. Due to a high E_2 level, the present authors consider this follicle to be mature.

For women, increasing age often means shorter menstrual cycles and a shorter follicular phases of menstrual cycle compared to women of child-bearing age. As a result, the dominant follicle will be selected earlier. Eventually, this will result in menopause. It has been illustrated that the earlier development, but not the faster growth, of dominant follicles is responsible for the earlier selection [11]. The patient in this report has been diagnosed with POR, so the earlier emergence of a dominant follicle is understandable. Previous research has shown that levels of FSH will rise before follicle recruitment [6], while older women always show earlier increases of FSH in luteal phase, probably due to the decrease of inhibit-A produced by corpus luteal, while a relatively strong negative correla-

tion was found between inhibit A and FSH level [12].

The pregnancy of the patient in this report provides some insights into infertility treatment. For patients with POR, every follicle is precious during a menstrual cycle. If there is chance to gain a mature follicle in the early follicular phase of the menstrual cycle, the attempt should not be abandoned. While for some cancer patients who need urgent fertility preservation, there is not enough time to conduct conventional hormonal ovarian stimulation which lasts two to six weeks. Furthermore, ovarian stimulation is always associated with relatively high levels of E_2 serum concentrations, which may be unsafe for some E_2-sensitive conditions in the opinion of many researchers [9]. Under these circumstances, mature oocytes appearing in early follicular phase of the menstrual cycle give hope to those who need urgent fertility preservation.

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