

Luteal-phase Ovarian Stimulation Case Report: Three-Year Follow-up of a Twin Birth

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Abstract

This unique study shows favorable outcomes to embryos developed from eggs retrieved following luteal phase stimulation and the children born from these eggs.

A 40-year-old woman with a 10-year history of primary infertility was given hMG 150IU and letrozole 2.5 mg from cycle day 3 onwards. Her cycle transitioned into the luteal phase beginning on cycle day 10 as indicated by high serum progesterone levels. Follicle growth continued with hMG stimulation. GnRH agonist was given for the final stage of oocyte maturation. During collection, four mature oocytes were obtained and two top-quality embryos were vitrified for cryopreservation. Two months later, the two embryos were thawed and transferred during a natural cycle, creating a twin pregnancy and a favorable delivery. Three-year follow up showed that physical and psychomotor development of the twin babies were in the normal range of children conceived naturally. This case report documents that embryos developed following luteal-phase ovarian stimulation reached viability, and child development was found to be normal up to three years following birth. This study opens the door to the possibility of successful outcomes from luteal phase stimulation. This has a great potential for patients who did not respond well during the follicular phase, but most importantly, for newly diagnosed cancer patients who need immediate fertility preservation, i.e., to quickly produce viable oocytes.

Keywords: Ovulation induction; Luteal phase; Human menopausal gonadotrophin; Letrozole; Frozen-thawed embryo transfer

Introduction

Ovarian stimulation is usually carried out with exogenous gonadotropins to induce multiple follicle development and to mature more oocytes for *in vitro* Fertilization (IVF) [1]. According to the traditional folliculogenesis, antral follicles in each ovary are recruited from the late luteal phase of the preceding menstrual cycle for the following follicle phase. The dominant follicle is selected during the beginning or the middle stage of the follicular phase, while the others undergo atresia [2]. The standard regimens of ovarian stimulation target the antral follicles in the follicular phase so gonadotropin stimulations always start during the early follicular phase of the menstrual cycle or within ten days of GnRH agonist administration [1].

However, some studies have demonstrated that the small antral follicles observed in the luteal phase may not necessarily be in atresia, but may rather be in the early stages of follicular development [3]. This indicates waves of follicular development within a single interovulatory period, with the presence of healthy follicles in the luteal phase as determined by oocyte and granulosa cell viability [3,4]. So it is possible that the follicles developing in the luteal phase have the potential to ovulate in the presence of an exogenous LH surge. Several researchers have reported that the protocols of luteal-phase ovarian stimulation or even random-start ovarian stimulation can be performed in the context of emergency fertility conservation and mature oocytes/embryos can be obtained for cryopreservation [5-10]. However, at present, no report has addressed the developmental competence of embryos originated from luteal-phase ovarian stimulation or random-start ovarian stimulation.

This paper reports the case of a patient diagnosed with primary infertility and given hMG 150IU and letrozole 2.5 mg from cycle day 3 onwards. Serum hormones showed sustained high progesterone levels after cycle day 10, indicating that patient's cycle had transitioned into the luteal phase. Follicle growth coincided with increased serum estradiol levels in the patient's luteal phase. Four mature oocytes were obtained, of which two viable embryos developed and implanted

following Frozen Embryo Transfer (FET). The successful pregnancy, delivery of twin babies and three years of follow up provided sound evidence for the developmental competence of the embryos originated following luteal-phase ovarian stimulation.

Patient Characteristics

A 40-year-old woman with a 10-year history of primary infertility and irregular menstrual cycle (of 23-29 days in length) and bilateral blocked tubes with one failed IVF attempt was referred to our unit.

Her endocrine evaluation revealed a FSH (follicle-stimulating hormone) level of 10.82 IU/l, LH of 10.5 IU/l, estradiol of 25 pg/ml and progesterone of 2.0 ng/ml. Her pelvic ultrasound scan showed three 5-mm-diameter antral follicles, an 11×4 mm ovarian cyst in the right ovary and no follicles in the left ovary.

The patient's partner's semen analysis was normal.

Treatment protocol

First cycle: During her first IVF cycle, the stimulation protocol included the use of clomiphene citrate (Codal Synto Limited, France) 50 mg/day from cycle day 3 and onwards, along with hMG (Anhui Fengyuan Pharmaceutical Co., China) 150 IU every other day. The peak estradiol level, which occurred on cycle day 12, was 736 pg/ml,

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Monitoring	Day number	3		10		16		19	
		L	R	L	R	L	R	L	R
FSH (IU/l)		8.5		14.8		9.3		8.8	
LH (IU/l)		2.5		6.3		0.6		0.7	
E2 (pg/ml)		87		56		208		507	
P (ng/ml)		0.2		2.1		19.2		6	
Follicle location		L	R	L	R	L	R	L	R
Follicle diameter (mm)		10		7		5		14	
		8		5		10		8	
						9		11	
						9		10	
						6			
						6			
Endometrial thickness (mm)		5		9		7		15	

Table 1: Monitoring by day.

with 2 follicles present - one 19 mm and the other 20 mm in diameter. GnRH agonist Decapeptyl 0.1 mg (Ferring GmbH, Germany) was given to induce final oocyte maturation. Transvaginal egg collection was performed 36 hours later. Two oocytes were aspirated, but due to polyspermia, no embryo was transferred.

Second cycle: Letrozole 2.5 mg was administered daily (Jiangsu Hengrui Medicine Co., China) along with hMG 150 IU, which was started daily on day 3 of the cycle. Only two small follicles appeared on the right ovary, with low estrogen serum concentration, 5 mm endometrium and normal serum FSH and LH (Table 1). On day 10, following 7 days of stimulation, follicle measurement showed that the previous group of medium-sized follicles had disappeared and a new cohort of antral follicles about 4 - 7 mm in diameter had emerged. Serum progesterone levels had increased to 2.1 ng/ml on cycle day 10. To our surprise, the progesterone level continuously increased to 19.2 ng/ml on cycle day 16, when an ultrasound showed two follicles - one 14 mm and the other 10 mm in diameter - two 9 mm-diameter follicles and two 6 mm-diameter. On day 19, progesterone levels fell to 6.0 ng/ml. The right ovary contained four follicles of more than 10 mm in diameter (21, 14, 11, and 10 mm in diameter, respectively) and endometrial thickness had increased to 15 mm. The Decapeptyl GnRH agonist 0.1 mg was administered, 7 oocytes were collected 36 hours later, and the oocytes were inseminated. Eighteen hours later, 4 oocytes were fertilized with two pronuclei, while one egg had abnormal fertilization, with three pronuclei, and two eggs remained unfertilized. On day 3, two 8-cell grade-1 embryos were vitrified and cryo-preserved for future transfer.

Two months following fertilization, during a natural cycle, FET was performed. On cycle day 12, a large follicle of 16 mm was detected; endometrial thickness was 10 mm. Ten thousand (10,000) IU of hCG (Lizhu Pharma Co, China) were administered, and four days later, 2 thawed embryos, were implanted. For luteal phase support, progesterone 60 mg IM daily was administered beginning at the time of transfer. A serum β -hCG level of 874 IU/l was detected 14 days after embryo transfer. A vaginal ultrasound performed 4 weeks later confirmed the presence of two intrauterine gestational sacs containing two viable fetuses.

Pregnancy follow-up

The twin pregnancy course was uneventful. A Caesarean section was performed at 35⁺² weeks, with a male infant born weighing 2800

g and measuring 49 cm, and a female infant weighing 2300 g and measuring 46 cm. After four days of observation, the babies and mother were discharged in good health. During the post-natal visit, mother and babies were well.

Twin development

The twin's physical and psychomotor development was observed by a pediatrician one year and three years after delivery. No malformations were diagnosed after birth. No special medical or surgical treatments or hospital visits were recorded. On examination, the twin babies' physical, language and psychosocial development were good. At 3 years of age, the boy weighed 18.5 kg and measured 102 cm in height, and the girl weighed 16.1 kg and measured 93 cm in height. Their sizes were in the normal range of children conceived naturally in China.

Discussion

This case report presents several interesting observations. The first is the presence of high progesterone levels from early stimulation process and lasting throughout the rest of it. Given the patient's history of irregular menstrual cycles and continuously increasing progesterone levels (up to 19.2 ng/ml at the later stage of ovarian stimulation, characteristic of the mid-term luteal phase), we concluded that treatment was performed during patient's luteal phase. One possible explanation is that patient's cycle had transitioned from the follicular phase into the luteal phase as evidenced by the continuously increasing progesterone level (2.1 ng/ml - 19.2 ng/ml) from cycle day 10 onwards. We also observed that two larger follicles of 10 mm and 8 mm in diameter, respectively, disappeared, with three smaller follicles presenting instead in the right ovary. We presumed that the first follicle wave had concluded and the second follicle wave began during the latter part of the stimulation process. The growth of this new cohort of follicles was accompanied by an increase in estradiol concentration. This observation is consistent with the theory of multiple follicular growth waves per cycle [4,11]. Sixty eight percent of women exhibited two waves of follicle development during one Interovulatory Interval (IOI), and 32% of the 50 healthy women observed exhibited three waves [4]. The luteal phase antral follicles do not grow as large, on average, as ovulatory follicles [4,11]. But, in the presence of an exogenous LH surge, the follicles developing during the luteal phase have the potential to mature and ovulate. The clinical outcome demonstrated that continuing high progesterone had no negative influence on follicle maturation and the quality of oocytes retrieved. The results supported that ovarian stimulation with letrozole and hMG during the luteal phase was feasible and safe for this patient. The second observation is the successful pregnancy outcome and the live birth of twin infants after a frozen-thawed embryo transfer. The previous studies reported that using luteal-phase ovarian stimulation or random-start ovarian stimulation can produce viable embryos, but no data addressed the pregnancy outcome and subsequent child development. As far as we know, this is the first report of live birth of healthy twin babies using embryos developed from luteal-phase ovarian stimulation. Three-year follow up demonstrated the physical and psychological well-being of the twin babies and found no difference from children who were conceived naturally. This case report provides evidence of the normal developmental competence of embryos originated during luteal-phase ovarian stimulation.

The third point regards the adjuvant usage of letrozole and hMG in luteal-phase ovarian stimulation. Letrozole, an aromatase inhibitor, has been demonstrated to be effective as an ovulation induction and controlled ovarian hyperstimulation agent. The efficient estrogen-lowering properties of aromatase inhibitors can be utilized to

temporarily release the hypothalamus from the negative effect of estrogen, thereby inducing an increased discharge of FSH [12]. The resulting accumulation of androgens may be a further advantage as androgens may have a stimulatory role in early follicular growth by augmenting follicular FSH receptor expression and therefore amplifying FSH effects [13-15]. However, administration has generally been limited to the early follicular phase. Is the role of letrozole in luteal-phase ovarian stimulation similar to its role in the follicular phase? These observations need to be confirmed by clinical trials and may lead to new thinking about ovarian stimulation management in assisted reproduction treatment.

This experience with luteal phase stimulation might have a major implication for patients diagnosed with cancer who wish to undergo fertility preservation, as this protocol will facilitate immediate stimulation no matter what stage in the cycle they are in. This application needs further support from clinical studies.

Conclusion

This case study demonstrates that embryos originating from follicles developed during the luteal phase had normal developmental competence, and that the protocol of luteal-phase ovarian stimulation with hMG and letrozole was able to produce competent oocytes/embryos, with optimal pregnancy outcome from the subsequent FET. Prospective, randomized trials are needed to verify these interesting findings. The study opens the door to extend the ovarian stimulation to routine IVF settings that can be utilized regardless of menstrual cycle, moving beyond the limitations of current IVF practices.

Authors' Contributions

Professor Kuang was the chief investigator. He supervised the entire case study procedure including concept, design and implementation and completion. Dr Chen, Dr Hong, Dr Lyu, Dr Ai and Dr Fu were responsible for data collection. Dr Chen and Dr Hong contributed to data analysis and drafted the manuscript. Professor Zeev Shoham contributed to the manuscript revision. All authors participated in the ultimate interpretation of the study data and manuscript editing.

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