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SHORT COMMUNICATION

# Live birth following early follicular phase oocyte collection and vitrified-warmed embryo transfer 8 days later

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**Abstract** A 30-year-old woman with premature ovarian insufficiency had two follicles measuring 17 mm and 14 mm on day 3 of her menstrual cycle. Serum oestradiol concentration was 210 pg/ml. Recombinant human chorionic gonadotrophin was given and 5 mg/day letrozole started orally. One metaphase II oocyte was collected 36 h later. A 4-cell embryo was vitrified on the second day after fertilization. Letrozole was stopped on cycle day 8 due to absence of any other visible antral follicles. Oestradiol valerate 6 mg/day was started and the endometrium was 9.2 mm on cycle day 11. The embryo was warmed and transferred on cycle day 13, the 8th day after oocyte retrieval. Luteal phase support with progesterone, oestradiol and low molecular weight heparin was started on the day of transfer and continued until the 10th gestational week. A healthy girl weighing 3200 g was born at term. Early follicular phase oocyte collection did not result in early opening of the implantation window. Apparently secretory transformation was not started until luteal phase support, enabling a cleavage stage embryo transferred 8 days later to implant. Either corpus luteum formation could be disrupted or the endometrium could remain unresponsive to progesterone during the early follicular phase.



**KEYWORDS:** aromatase inhibitor, endometrial receptivity, implantation, menstrual cycle, premature ovarian insufficiency, random start stimulation

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## Introduction

Premature ovarian insufficiency (POI) is early onset ovarian malfunction and affects 1–3% of reproductive age women. Serum follicle stimulating hormone (FSH) concentrations in the postmenopousal range, i.e. ≥40 IU/l, is diagnostic for POI in a woman aged <40 years with normal 46 XX karyotype. Most patients present with irregular spontaneous menstrual cycles; therefore amenorrhoea is not essential for definitive diagnosis of POI (Nelson, 2015).

Women with POI frequently seek fertility-promoting therapies. However, they often respond poorly or do not respond at all to ovarian stimulation, resulting in a poor prognosis. There is no consensus on the optimal stimulation protocol for women with POI. It has recently been shown that follicular growth can be induced at any time of the menstrual cycle, and this is called random start ovarian stimulation (Cakmak et al., 2013). Oocyte donation studies showed that endometrium can be maintained on oestrogen for up to 6 weeks without an adverse affect on implantation (Remohí et al., 1995). Hence, it could be possible to manage ovarian and endometrial cycles separately during an assisted reproductive technology (ART) cycle. Exploiting these two facts, we were able to achieve an ongoing pregnancy with vitrified/warmed embryo transfer 8 days after oocyte retrieval in a woman with POI.

## Case report

A 30-year-old woman presented with a 2-year history of primary infertility. Apart from irregular menstrual cycles ranging from 60 to 120 days, her medical history was unremarkable. Her serum FSH values ranged between 49 and 150 IU/l, measured on two occasions >4 weeks apart, and her serum anti-Müllerian hormone value was <0.1 ng/l. She had a normal 46 XX karyotype. There was no other endocrinologic abnormality except for elevated serum thyroid-stimulating hormone concentration at 8.55 IU/l.

Pelvic examination did not reveal any abnormal findings. Transvaginal ultrasound showed only one antral follicle, an apparently normal uterus and a thin endometrial lining. Her partner had a normal semen analysis. She was diagnosed with POI and offered ART following further investigaton and treatment of her hypothyroidism. Her serum free T4 concentration was low and she was given levothyroxin orally. The dose was adjusted to keep serum thyroid-stimulating hormone concentrations below 2.5 IU/l. She refused screening for Fragile X premutation status.

Ovarian stimulation with 5 mg/day letrozole (Femara tablet; Novartis Pharma, Switzerland) and 150 IU/day menotropin (Merional; IBSA Pharmaceuticals, Switzerland) commenced on the third day of a spontaneous period and resulted in the collection of a metaphase II oocyte. Despite normal fertilization with intracytoplasmic sperm injection (ICSI), development was arrested before cleavage. Five similar stimulation cycles were all cancelled due to the absence of follicular growth following 7 days of stimulation.

Three months after the sixth attempt she presented on the third day of a spontaneous period. Transvaginal ultrasound showed two follicles of 17 and 14 mm, a thin endometrium, and her serum oestradiol concentration was 210 pg/ml. Given the possibility of rapidly growing follicles, 250 μg human chorionic gonadotrophin (HCG; Ovitrelle, MerckSerono, Switzerland) was administered subcutaneously and letrozole 5 mg/day was started simultaneously. Thirty-six hours later one metaphase II oocyte was collected and succesfully fertilized with ICSI. A 4-cell embryo was vitrified on the second day after fertilization. Letrozole was stopped on cycle day 8, i.e. 3 days after oocyte retrieval, due to the absence of any visible antral follicles. It was decided to abandon stimulation and proceed with the transfer of the cryopreserved embryo in the same cycle. Oestradiol valerate (Estrofem tablet; Novo Nordisk, Denmark) 2 mg every 8 h orally was started on the same day. Endometrial thickness was 9.2 mm on cycle day 11. Oestradiol dose was increased to 8 mg/day and vitrified-warmed embryo transfer was scheduled for 2 days later. On cycle day 13, all four blastomeres survived warming and the embryo was transferred under ultrasound guidance 8 days after oocyte retrieval. Oestradiol valerate was continued at the same dose of 8 mg/day and luteal phase support (LPS) with 100 mg/day progesterone in oil (Progynex 50 mg/ml; Koçak Farma, Turkey) intramuscularly and 0.4 mg/day low molecular weight heparin (Clexane; Sanofi Aventis Intercontinental, France) subcutaneously, was started on the day of transfer. Management of the cycle is shown in **Figure 1**.

Serum β-hCG concentration was 149 mIU/ml on the 12th day after embryo transfer and 384.1 mIU/ml 2 days later. A singleton intrauterine pregnancy with a heartbeat was visualized 2 weeks later. LPS was continued until the 10th gestational week. The rest of the pregnancy was uneventful and she gave birth to a healthy girl weighing 3200 g at 39 weeks.

Koç University Committee on Human Research approved the publication of this case report on 4 August 2015.

## Discussion

POI impairs reproductive function years before normal menopause. Various protocols have been tried to improve ovarian response to stimulation but they fail to do so in the majority of cases (Nelson, 2015). Traditionally stimulation starts in the early follicular phase, when the antral follicles are still small. However, in contrast to the notion of a single follicular recruitment episode during one menstrual cycle, multiple cohorts or 'waves' of antral follicle recruitment have been described (de Bianchi et al., 2010). A follicular wave is defined as the synchronous growth of a group of antral follicles that occurs at regular intervals during the menstrual cycle. Distinct follicular waves have been recently detected in women during the perimenopausal transition (Hale et al., 2007) and in women undergoing ovarian stimulation therapy (Bentov et al., 2010). Elevated circulating FSH appears to precede the recruitment of each follicular wave during the interovulatory interval. High endogenous FSH concentrations during luteo-follicular transition can cause follicles reaching pre-ovulatory status earlier in the subsequent cycle. This is not an uncommon occurrence in POI, as indicated by detection of high serum oestradiol concentrations, i.e. >65–80 pg/ml, as early as the third day of the menstrual cycle. Indeed, elevated serum oestradiol concentrations in the early follicular phase is a sign of 'occult ovarian failure'. While such early growing follicles were regarded as 'functional cysts'

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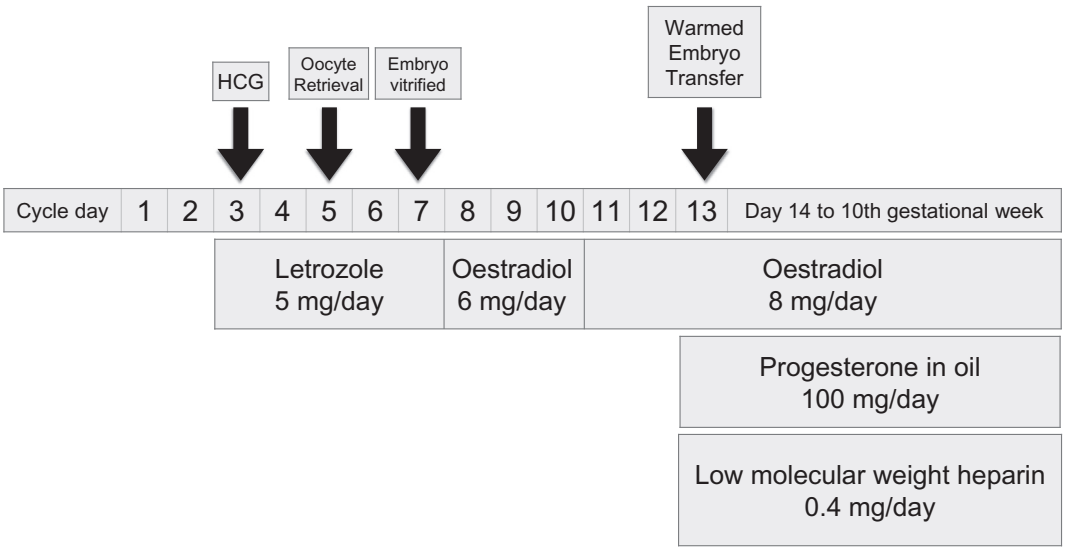


**Figure 1** Depiction of medications and procedures during the treatment cycle.

requiring cancellation of treatment or aspiration before starting stimulation in the past, it is recently recognized that these follicles can harbour competent oocytes that can be fertilized *in vitro*. It is possible to harvest mature oocytes from them following HCG administration right away if they have already reached pre-ovulatory size or following a short course of stimulation until they reach pre-ovulatory size if they are smaller. In such cases, serum oestradiol concentration is used as a marker of follicular growth and we regard concentrations over 150 pg/ml reassuring for proceeding with HCG administration and oocyte retrieval regardless of the cycle day. In the present case we simultaneously started ovarian stimulation with letrozole in the hope that another follicular wave following oocyte retrieval would occur. In dual stimulation cycles, the second round of gonadotropins are usually started after oocyte retrieval; however, in those series the first oocyte retrieval already followed a stimulation period, unlike the present case. Although the absence of other antral follicles visible on ultrasound was discouraging, letrozole is an inexpensive stimulation agent that is taken orally; therefore it was thought it would be appropriate to start it before oocyte retrieval despite the risk of being ineffective. Indeed, as there were no growing follicles after 5 days on letrozole, stimulation was cancelled.

An unusual aspect of the present case is the implantation of the embryo transferred 8 days after oocyte retrieval in the same cycle. Progesterone secreted by the corpus luteum, which would have formed after oocyte retrieval, could be expected to lead to secretory transformation of the endometrium and opening of the 'implantation window'. In that case, the implantation window would have already been closed by the time the cleavage stage embryo, which was transferred eight days after oocyte retrieval, would become a hatching blastocyst after an additional 3–4 days, i.e. 11–12 days after oocyte retrieval and corpus luteum formation. However, rather than successful implantation occurring after closure of the implantation window, it is more likely that the implantation window has been shifted further in the cycle by one or more of the mechanisms described below.

Perhaps corpus luteum formation is disrupted in women with POI, preventing adequate progesterone production, which would be required to induce secretory changes in the endometrium. Another possible explanation would be endometrial development being inadequate for secretory transformation by cycle day 5, i.e. just 1–2 days after cessation of menstrual bleeding, despite the presence of progesterone. It is well known that endometrial progesterone receptor expression, hence progesterone effect, requires oestrogen priming (Kreitmann et al., 1979). Letrozole administration between cycle days 3 and 7 may have also facilitated prevention of secretory transformation before embryo transfer in the present case. Although inhibition of aromatization could have led to accumulation of progesterone together with other precursors of oestrogens, it could also have prevented adequate oestrogen priming of the endometrium to induce secretory changes. This could enable the endometrium achieving receptivity later on, upon exposure to exogenous progesterone as LPS, i.e. the implantation window being shifted. In our opinion the latter explanation is more likely and it formed the rationale of our management in this case. It was decided to proceed with the transfer of the cryopreserved embryo in the same cycle upon observing a trilinear endometrium, suggesting lack of secretory transformation, despite several days following oocyte retrieval. The main limitation of this report is that sequential progesterone measurements were not available. Unfortunately, as a private hospital, not associated with a research unit, we were unable to measure serum progesterone or luteinizing hormone concentrations daily at the time of treatment or by the use of frozen samples.

Low molecular weight heparin was empirically included in the luteal support protocol by the treating physician, based on the limited evidence suggesting a beneficial effect on implantation process (Tersigni et al., 2012).

In conclusion, we report the implantation of a cleavage stage embryo, derived from an oocyte collected on the fifth day of a menstrual cycle, transferred eight days after oocyte retrieval. This provides further evidence that large follicles

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present in the early follicular phase should not be neglected and endometrial receptivity may not be impaired with early oocyte collection in ART cycles. Early follicular phase oocyte retrieval is increasingly done, especially for poor responders. Contrary to the general practice of a freeze-all and transfer in a later cycle strategy, it may be feasible to proceed with cryopreserved transfer, fresh transfer, if a second oocyte retrieval could be done following dual stimulation, or a combination of both in the same cycle.

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