Metaphase II (MII) oocytes obtained at different time points in the same in vitro fertilization cycle

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Introduction

Oocyte maturation is required to complete meiosis and to produce a competent oocyte able to sustain embryo development, implantation and pregnancy. In humans, meiosis begins in the developing ovaries at 11–12 weeks of gestation [4]. The oocytes progress to diplotene stage of prophase I and then arrest until ovulation, an event that can occur decades later. Oocytes resume meiotic maturation in response to the midcycle luteinizing hormone (LH) surge. This final oocyte maturation can be induced medically with either human chorionic gonadotropin (hCG) or a gonadotropin-releasing hormone agonist (GnRHa). The former binds the LH receptor, while GnRHa promotes the release of endogenous gonadotropin stores from the hypophysis.

The resumption of meiosis (so called nuclear maturation) occurs 14–18 h after the beginning of the LH surge; meiosis I is completed within 35 h and the oocytes reach the metaphase II stage (MII) [16]. Meanwhile, in response to the LH surge, the cumulus cells display almost complete expansion by 20 h; then the mature cumulus-oocyte complex detach from the follicular wall before ovulation [2]. While nuclear maturation and cumulus expansion are closely linked, it is unclear if different LH levels are needed to regulate the two processes and if different follicles have different LH thresholds.

Capsule A case presentation in which MII oocytes were obtained at two different time points within an IVF cycle after GnRH agonist and subsequent hCG triggers.

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In this report, we describe a case of an oocyte donor who received a GnRHa trigger that resulted in low number of retrieved oocytes; all oocytes had minimally expanded cumulus cells but showed evidence of nuclear maturation (MII). Due to low oocyte yield, the patient received an hCG trigger on the evening of this first retrieval. Repeat retrieval 34 h after hCG resulted in recovery of an appropriate number of mature oocytes.

Case presentation

A 22-year-old nulliparous woman, with a history of regular menstrual cycles and a negative medical/family history, underwent an anonymous oocyte donor cycle. She was using combined oral contraceptives at time of her initial screening. Before ovarian stimulation, her antral follicle count was 21 and anti-mullerian hormone level was 11.75 ng/ml.

Stimulation was performed with follicle-stimulating hormone (FSH) 150 IU/day (Follistim, Organon, USA) and human menopausal gonadotropin 150 IU/day (hMG) (Menopur; Ferring Pharmaceuticals, USA) subcutaneously. The starting dose of medication was based on her antral follicular count [11] and not changed throughout the cycle. A GnRH antagonist (Ganirelix; Organon: 0.25 mg/day subcutaneously) was added on cycle day (CD) 9 when the lead follicle reached 12 mm, and it was continued until the day of ovulation induction. On CD 13, oocyte maturation was triggered with a subcutaneous injection of 1 mg of leuprolide acetate (Lupron; Abbott Laboratories, USA) when the two lead follicle were ≥ 18 mm in size and serum estradiol level was 4,519 pg/ml. There were 22 follicles ≥ 13 mm on the day of GnRH trigger. Twelve hours after the leuprolide acetate injection, serum LH level was 17.7 mIU/ml. Transvaginal ultrasound-guided oocyte retrieval was performed 36 h after leuprolide acetate administration (on CD15). At the time of

oocyte retrieval 10 dominant follicles were aspirated from the right ovary, but only three oocytes with minimally compacted cumulus cells surrounding were obtained (Table 1). The retrieval was stopped due to low oocyte yield. Serum LH level was repeated and found to be 2.35 mIU/ml. The three retrieved oocytes were immediately stripped and all 3 were noted to have completed nuclear maturation (MII).

The decision was made to administer 10,000 IU of hCG (Organon) on the evening of the same day (CD15) and to repeat follicular aspiration 34 h later. On CD16, urine hCG was positive, serum LH level was 0.46 mIU/ml, estradiol level was 5,525 pg/ml and progesterone was 39 ng/ml. The second transvaginal retrieval was performed on CD 17 and 39 oocytes were retrieved (13 from the right ovary). Twenty-nine of the 39 oocytes had evidence of nuclear maturation (MII stage).

All MII oocytes were inseminated by the ICSI procedure. Two of the three from the first retrieval and 22 of the 29 MII oocytes from the second retrieval fertilized normally with visualization of 2 pronuclei. Two day 2 embryos (grades: 232 and 422) generated from the first oocyte retrieval were transferred to the recipient. Pregnancy was not achieved. Twenty-two 2PN embryos originated during the second oocyte retrieval were cultured for 3 days; nine day 3 embryos (grades: 1033, 833, 822, 821, two 722, two 733 and 633) are currently frozen for future use.

On CD 21, the patient presented with symptoms of abdominal distention and discomfort, nausea, vomiting and inability to tolerate food orally. Evaluation showed large amount of ascites, intravascular volume depletion, and electrolyte abnormalities (hyponatremia and hyperkalemia) consistent with moderate to severe OHSS. The patient was hospitalized for intravenous fluid resuscitation, correction of electrolyte abnormalities, pain management and control of nausea and vomiting. The patient had paracentesis on two occasions for symptomatic relief during her hospital stay. On CD 27, patient's electrolyte levels normalized, ascites improved and the patient was discharged home.

Discussion

Herein we present a case in which MII oocytes were obtained at two different time points within an IVF cycle after GnRHa and subsequent hCG triggers.

Table 1 Summary of oocyte yield and maturation in oocyte retrievals

	# of Follicles Aspirated	# of Oocytes Retrieved	MI oocytes	Cumulus cells	2PN
1st retrieval	10	3	3	Minimally expanded	2
2nd retrieval	~40	39	29	Expanded	22

This case suggests that different follicles may have different LH thresholds for cumulus expansion and oocyte maturation. In particular, the retrieval of MII oocytes following the initial GnRHa trigger is somewhat surprising. In fact, if inadequate LH was produced following the GnRHa trigger, we would have expected either no oocytes being retrieved or retrieval of only immature (GV state) oocytes. The immediate cumulus cell stripping following oocyte retrieval for the ICSI procedure indicates that MII oocytes were not the result of extended culture in vitro.

To our knowledge this is the first report to suggest different LH levels may be needed for cumulus expansion and nuclear maturation. Both the low oocyte number retrieved (3 oocytes from 10 dominant follicles) and the compaction of the cumulus following the first retrieval suggest that the cumulus cells were not exposed to a sufficient LH dose. In contrast, the fact that all 3 oocytes were at MII stage indicates that the LH level was sufficient for nuclear maturation. The second retrieval resulted in an expected number of oocytes retrieved with appropriate cumulus expansion and nuclear maturation (29 out of 39 oocytes were MII). The immature oocytes were likely the result of aspiration of small follicles (10 mm) [12].

It is possible that different LH surge dynamics induced by GnRHa are responsible for our findings. The injection of GnRHa results in an acute release of LH and FSH. Serum LH and FSH levels rise for 4 and 12 h, respectively, and remain elevated for 24 to 36 h [9]. The amplitude of the LH surge with GnRHa is similar to that seen in the normal menstrual cycle, but has a different shape and duration. The natural LH surge is characterized by three phases with a total duration of 48 h: a rapidly ascending limb of 14-h duration, a plateau of 14 h, and a descending phase of 20 h [13]. On the contrary, the GnRHa induced LH surge consists of two phases with duration of 24–36 h: a short ascending limb (>4 h) and a long descending limb (>20 h) [6,9]. This leads to a significantly reduced total amount of LH and FSH released from the pituitary when GnRHAs are used to trigger final oocyte maturation. As a consequence, corpus luteum deficiency and luteal phase defect are possible [8]. Given its significantly longer half-life (>24 h versus 60 min for LH), hCG has a prolonged luteotrophic effect causing the development of multiple corpora lutea and significantly higher serum and follicular fluid steroid levels [1,5]. It is therefore possible that the shorter duration and lower amount of LH released after GnRHa trigger may not be sufficient to induce both nuclear maturation and cumulus cells expansion in all patients.

Another possibility is that dyssynchronous follicular growth was responsible for these findings. However, we consider the lack of synchronization of the follicles hypothesis less likely since the pattern of the follicular growth of the patient was optimal and comparable to the response typically

seen in a controlled ovarian stimulation cycle: In particular the follicles at time of initial trigger showed a balanced continuity from large to small size. Furthermore only large follicles of similar size were aspirated at the time of the first retrieval.

The second information gathered from this case is that different patients may have different responses to a GnRHa trigger. In our case, the LH level 12 h post-GnRHa-trigger was 17.7 mIU/ml and was insufficient to induce oocyte maturation in all follicles. Importantly the same dose (1 mg) of GnRHa was used in numerous other patients in our practice and resulted in appropriate LH release. The correlation between serum LH level at 12 h post-trigger with GnRHa and oocyte yield/maturity has been previously assessed [3,15]. Shapiro et al. demonstrated that LH level at 12 h post-trigger <52.0 mIU/ml was suboptimal with the risk of submaximal oocyte yield and maturity [15]. In the same study, LH criterion of <12.0 mIU/ml provided a 50 % sensitivity and 97 % specificity in predicting low yield and achieved 38 % sensitivity and 97 % specificity in predicting low maturity [15]. Similarly, Chen et al. showed that LH levels <15.0 mIU/ml were inadequate for oocyte yield [3]. However, the authors did not observe a decline in oocyte maturity with low LH levels. Finally, in a previous case report, GnRHa trigger resulted in no oocytes being recovered in two patients with polycystic ovary syndrome [7].

GnRH antagonists have higher affinity to GnRH receptor compared to GnRHa and only a portion of GnRH antagonist is displaced by GnRHa when administered [10]. Therefore, one solution to facilitate a proper LH surge post-GnRHa trigger is to administer a higher dose of GnRHa. In our current practice, 4 mg of leuprolide acetate is being used effectively with optimal oocyte yield and maturation. Another option is to combine the biological activity of LH and hCG. Low-dose hCG (1000–2500 IU) concomitant with GnRHa has been tried with apparent success as a “dual trigger” [14]. There is however, a clear need to perform more studies to identify the appropriate dose of GnRH agonists required for a fully optimal LH surge.

In conclusion, this case suggests that different follicles in the same patient may have different LH thresholds for oocyte maturation and cumulus expansion. From a clinical standpoint, this case indicates that if a low number of oocytes are recovered following GnRHa trigger, it is possible to trigger ovulation again by administering hCG.

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Conflict of interest statement The authors declare that there are no conflicts of interest.

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