CASE REPORT

Ovulation by repeated human chorionic gonadotrophin in ‘empty follicle syndrome’ yields a twin clinical pregnancy

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This case illustrates the possibility of obtaining oocytes that fertilize and cleave normally after the administration of a second ovulatory dose of human chorionic gonadotrophin (HCG) in a case of ‘empty follicle syndrome’. The present patient underwent ovarian stimulation with gonadotrophin-releasing hormone analogue (GnRHa)/menotrophins for intracytoplasmic sperm injection (ICSI). After the failure of the first oocyte retrieval, a second dose of HCG was administered to trigger ovulation. A total of 13 oocytes was retrieved during the second procedure and 11 good quality embryos were obtained (fertilization and cleavage rates of 92 and 91% respectively). No pregnancy was achieved after the replacement of three embryos. In a subsequent cycle stimulated with clomiphene citrate, three frozen–thawed embryos were replaced and a twin pregnancy was achieved. The patient delivered two healthy babies at term.

Key words: empty follicle syndrome/intracytoplasmic sperm injection/ovarian stimulation

Introduction

The failure of oocyte retrieval after repeated aspiration and flushing of mature follicles during oocyte retrieval for assisted reproductive techniques is a rare event defined as empty follicle syndrome (EFS) (Coulam et al., 1986). Some authors have reported subnormal concentrations of progesterone and luteinizing hormone (LH) in follicular fluid of patients with EFS (Phocas et al., 1992). However, a clear explanation of this phenomenon has not yet been proposed. Recently, Zegers Hochschild et al. (1995) suggested that EFS is not due to an ovarian problem but a result of an abnormal in-vivo bioactivity of some batches of commercially available human chorionic gonadotrophin (HCG). In the present case, the replacement of frozen–thawed embryos obtained from oocytes retrieved after a second HCG injection yielded a twin ongoing pregnancy in a woman with EFS who underwent ovarian stimulation with gonadotrophin-releasing hormone analogue (GnRHa) and menotrophins for intracytoplasmic sperm injection (ICSI).

Case report

The patient was a 26 year old woman married to a man who underwent radiotherapy to treat a testicular seminoma in 1987. Before radiotherapy treatment he provided two sperm samples which were frozen. The ovarian function was normal as judged by serial measurement of follicular stimulating hormone (FSH), LH, 17-β oestradiol and progesterone. The karyotype of both partners was normal.

Ovarian stimulation was carried out using buserelin intranasal spray (6×100 µg/day) in a desensitizing protocol started on day 21 of the luteal phase of the previous menstrual cycle. When oestradiol concentrations were <40 pg/ml and no ovarian cystic structures were observed at ultrasound examination, ovarian stimulation was started with human menopausal gonadotrophin (HMG). From day 6 of the stimulated cycle onwards, blood samples were taken and assayed for oestradiol, progesterone, LH and FSH. On days 10 and 11 of the follicular phase, progesterone serum concentrations were respectively 1.2 and 1.8 ng/ml despite suppressed serial LH concentrations. On day 11, oestradiol concentration was 3451 pg/ml and 21 follicles ≥16 mm diameter were detected by vaginal ultrasonography. Although the basal hormonal profile was normal, the patient had an exaggerated response to the 112.5 IU daily dose of HMG. An ovulatory dose of 10 000 IU HCG was administered (day 0). The serum HCG and progesterone concentrations were respectively 51 mIU/ml and 2.7 pg/ml 12 h later (day +1). At 36 h after HCG administration, oocyte retrieval was carried out by ultrasound-guided transvaginal aspiration. No cumulus–corona cell complexes and very few granulosa cells were retrieved after repeated aspiration and flushing of eight follicles (four from the right and four from the left ovary) with a diameter between 18 and 22 mm. The oocyte retrieval was then interrupted and a second dose of 10 000 IU HCG from a different batch was administered. The serum HCG and progesterone concentrations were respectively 51 mIU/ml and 2.7 pg/ml 12 h later (day +1). At 36 h after HCG administration, oocyte retrieval was performed by ultrasound-guided transvaginal aspiration. No cumulus–corona cell complexes and very few granulosa cells were retrieved after repeated aspiration and flushing of eight follicles (four from the right and four from the left ovary) with a diameter between 18 and 22 mm. The oocyte retrieval was then interrupted and a second dose of 10 000 IU HCG from a different batch was administered. The serum HCG and progesterone concentrations were respectively 253 mIU/ml and 40 pg/ml 20 h later (day +3). A few months earlier in a similar case, a second dose of HCG was administered and the second oocyte retrieval was performed 36 h later. Mature oocytes were retrieved and fertilized normally after ICSI procedure (unpublished observations). In the present case, since serum progesterone concentration on the day of the first oocyte retrieval was already 12 ng/ml (day +2), we were reluctant to wait 36 h for the second oocyte
retrieval, which was planned 24 h after the second HCG administration. A total of 13 cumulus–corona cell complexes was retrieved at the second oocyte retrieval from about 20 follicles with diameters between 14 and 25 mm. On the day of the second oocyte retrieval (day +3) the serum oestradiol concentration was 8780 pg/ml, whereas 2 and 3 days later (days +5 and +6) the serum oestradiol and progesterone concentrations were 2012 pg/ml and 53 ng/ml, and 2531 pg/ml and 58 ng/ml respectively. No more blood samples were taken during the mid-luteal phase.

After the enzymatic and mechanical removal of the cumulus and corona cells, all of the 13 oocytes were in metaphase I stage. Six of them were put in co-culture with granulosa cells in B2 Ménézo medium (BioMérieux, Lyon, France) and the other seven oocytes were put in co-culture with Vero cells in B2 Ménézo medium (bioMérieux, Lyon, France). After 18 h of in-vitro maturation all of the 13 oocytes extruded the first polar body and the ICSI procedure was performed (Van Steirteghem et al., 1993).

After thawing one vial of 1 ml, the sperm concentration was 11×10⁶/ml with a progressive motility of 5% and a normal morphology of 1%. In all, 12 oocytes were normally fertilized and 11 good-quality embryos (<20% anucleate fragmentation) were obtained. Three embryos, respectively 7-cell, 8-cell and 9-cell, were transferred into the uterus (day +6) about 56 h after ICSI procedure whereas eight 5- to 8-cell embryos were cryopreserved. The luteal phase was supported by means of vaginally administered micronized natural progesterone (600 mg/day). The serum HCG concentration detected 11 days after the embryo transfer was <5 mIU/ml and this negative result was confirmed 2 days later.

Two months later, the eight frozen embryos were thawed; four of them survived the procedure and the three embryos of the best quality (two eight-cell and one ten-cell embryo) were replaced directly after thawing during a cycle stimulated with clomiphene citrate. The patient received luteal phase support by means of HCG (2000 IU/day administered every 4 days starting from the day of embryo transfer and for three times) and vaginally administered micronized natural progesterone (600 mg/day). No blood samples were taken during the luteal phase of this cycle. The pregnancy was confirmed by a serial rise in serum HCG concentrations on two consecutive occasions 14 days after embryo replacement. Five weeks after embryo transfer two gestational sacs with positive heart activity were detected by means of ultrasound. The patient delivered two healthy children at term.

Discussion
It is a common experience of in-vitro fertilization (IVF) teams to have, at least once, experienced a ‘no oocyte retrieval’ in patients showing normal follicular development at ultrasound. This condition was defined as empty follicle syndrome, but a clear explanation of this event has not yet been given. During the last year we observed one patient undergoing ovulation induction for ICSI with a normal follicular development and a previous normal oocyte retrieval in which no cumulus–corona cell complexes and very few granulosa cells were retrieved after repeated aspiration and flushing of few mature follicles (unpublished observations). The suspicion of a lack of exposure or an insufficient response to biologically active HCG led us to stop the oocyte retrieval and to administer a second dose of HCG. A second oocyte retrieval was performed 36 h later and metaphase II stage oocytes were retrieved. Normal fertilization and cleavage were obtained. Similar cases of EFS were recently confirmed by Zegers–Hochschild et al. (1995).

In the case reported here, we were reluctant to wait 36 h after the second HCG administration for the oocyte retrieval because on that day serum progesterone concentration was already 12 pg/ml and we were afraid that spontaneous ovulation could occur if oocyte retrieval was planned 36 h after the administration of the second dose of HCG. At that moment none of the oocytes had yet extruded their polar body. Maybe if we had waited 12 h more, spontaneous ovulation would not have occurred and we might have been able to obtain metaphase II stage oocytes.

Two hypotheses for the absence of oocytes in this patient can be put forward. Either the dose of HCG injected had insufficient bioactivity or the ovaries showed an insufficient or delayed response to the administered dose. The fact that serum progesterone had risen to a value of 12 ng/ml 36 h after HCG administration is an argument against the biological inactivity of the injected preparation. The alternative hypothesis of a lack of response to HCG due to insufficiently matured granulosa–cumulus cells should also be considered. It could be possible that in patients showing an ovarian hyper-response, the expression of LH receptors was delayed or was insufficient at the moment of HCG injection.

The fertilization and cleavage rates of the metaphase II stage oocytes obtained after co-culture with granulosa and Vero cells were 92 and 91% respectively. This might indicate that in-vitro maturation of immature oocytes provides an effective solution when oocyte retrieval is performed earlier than 36 h after HCG administration.

The embryo transfer was performed on day +6, 6 days after continuous endometrial exposure to very high progesterone concentration (mean 20.8 ng/ml). Navot et al. (1991) in their original study in the in-vivo model of embryo donation demonstrated that implantation occurs when 2–3 day old embryos are transferred between luteal day +2 to luteal day +6 (cycle days 16–19 of a normal 28 day cycle). However, in this patient serum progesterone rise started on follicular day –1, and in a recent study we observed that serum progesterone rise during the follicular phase may induce a statistically significant endometrial advancement compared to patients with normal serum follicular progesterone concentration (<1 ng/ml), which is not related either to the number of days of progesterone rise or to the cumulative exposure of progesterone (Ubaldi et al., 1997). Taking these considerations into account, it is possible that the embryo transfer occurred outside the implantation window.

When three frozen–thawed embryos were replaced in a subsequent cycle stimulated with clomiphene citrate, two embryos implanted, resulting in an ongoing twin pregnancy.

In conclusion, this case report would suggest: (i) the empty
follicle syndrome might be the result of a lack of exposure to biologically active HCG or an insufficient end-organ response to biologically active HCG; (ii) an extra dose of 10 000 IU HCG might allow the retrieval of oocytes which fertilize and cleave normally; (iii) implantation might be hampered in a particular cycle by long exposure of the endometrium to high serum progesterone concentration; (iv) frozen–thawed embryos replaced in a subsequent cycle can implant normally, resulting in ongoing pregnancies.

References

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