CASE REPORT

Recurrent empty follicle syndrome successfully treated with recombinant human chorionic gonadotrophin

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We report a case of a patient with polycystic ovary syndrome and primary infertility who was admitted to our in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) programme because of her partner’s severe oligozoospermia and asthenozoospermia. Ovarian stimulation was accomplished in the three treatment cycles using gonadotrophin therapy after a dual approach with ovarian suppression using oral contraceptive pills followed by gonadotrophin-releasing hormone agonist therapy. Oocyte retrieval was unsuccessful in the first two treated cycles despite the fact that human chorionic gonadotrophin (HCG) from three different batches was used. In the third treatment cycle, recombinant HCG was used and five oocytes were retrieved. This is the first report of recurrent empty follicle syndrome despite the use of different batches of commercially available urinary HCG, and of its successful treatment using recombinant HCG.

Key words: empty follicle syndrome/HCG/in-vitro fertilization/recombinant gonadotrophins/recombinant HCG

Introduction

The empty follicle syndrome (EFS) (Coulam et al., 1986) is characterized by the lack of retrieved oocytes from follicles after ovulation induction and apparently normal follicular development for in-vitro fertilization (IVF), despite repeated aspiration and flushing. The problem occurs in 0.5–2% of IVF cycles according to recent reported series (Ben-Shlomo et al., 1991; Zegers-Hochschild et al., 1995; Quintans et al., 1998). The syndrome is considered a sporadic event with most reported cases having successful oocyte retrieval in previous and/or subsequent treatment cycles, thus implying that the condition is related to abnormal terminal follicular developmental events during the cycle in question (Ben-Shlomo et al., 1991; Zegers-Hochschild et al., 1995; Meniru and Craft, 1997). In fact, the recurrence of the syndrome in the same patient has been described only very occasionally and no treatment has been reported in such cases (Coulam et al., 1986; Rudak et al., 1990; La Sala et al., 1991). However, EFS has been cured in the same cycle when oocytes were not obtained from follicles in one ovary and a second injection of human chorionic gonadotrophin (HCG) from a totally different batch yielded retrieved oocytes from the other ovary 36 h later (Ndukwe et al., 1997; Ubaldi et al., 1997). This would imply that EFS is a drug-related problem rather than a clinical dysfunction.

This report represents the first case of recurrent EFS, despite the use of different batches of commercially available urinary HCG, and its successful treatment using recombinant HCG.

Case report

The patient was a 31 year old woman with polycystic ovary syndrome and primary infertility of 5 years’ duration who was admitted to our IVF/ICSI programme because of her partner’s severe oligozoospermia and asthenozoospermia. She had oligomenorrhoea (usually anovulation alternating with sporadic ovulatory cycles with delayed ovulation as confirmed by basal body temperature and mid-luteal plasma progesterone concentrations), her body mass index was 33, the basal luteinizing hormone/follicle stimulating hormone (LH/FSH) ratio was 4, and ultrasonographic scanning revealed the appearance of polycystic ovaries (Adams et al., 1986). Endogenous oestrogen activity was evident in the patient by basal oestradiol concentrations of 129 pg/ml and a positive response to a progestin challenge test (normal withdrawal bleeding after treatment with oral medroxyprogesterone acetate, 10 mg daily for 5 days). Hysterosalpingography, the karyotype of both partners and the DAZ gene in the male were all normal.

Ovarian stimulation was accomplished in three treatment cycles using gonadotrophin therapy after a dual approach with ovarian suppression using oral contraceptive pills followed by gonadotrophin-releasing hormone (GnRH) agonist therapy as previously reported by others (Rosenwaks et al., 1996). The oral contraceptive pill was given for 21 days. Leuprolide acetate was begun on day 17 at a daily dose of 1 mg s.c., overlapping the oral contraceptive pill for 4 days and continued until the administration of HCG. Gonadotrophin stimulation of the ovaries was started after 14–16 days of s.c. leuprolide when serum oestradiol concentrations declined to <50 pg/ml and a vaginal ultrasonographic scan showed an absence of follicles >10 mm in diameter.

In the first treated cycle, ovarian stimulation was carried out using highly purified FSH (Neo-Fertinorm®, Serono SA,
Madrid, Spain) which was administered for 8 days (300 IU s.c. daily for 2 days and then 75 IU daily for 6 days). HCG (Profasi\textsuperscript{®}; Serono) 5000 IU was given on day 9 of stimulation when a total of 20 follicles was present, with 14 being \( \geq 14\) mm in diameter and the three leading follicles measuring \( \geq 18\) mm (oestradiol concentration 1703 pg/ml; endometrial thickness 10 mm). Thirty-six hours later all of the available follicles were transvaginally aspirated and flushed repeatedly under sonographic control but no oocytes were recovered despite the presence of normal follicular fluid and granulosa cells in some follicular samples. The \( \beta\)-HCG serum concentration on the day of oocyte retrieval was 46 IU/l.

In the second treatment cycle the same approach for pituitary–ovarian suppression was used but recombinant FSH (Gonal-F\textsuperscript{®}; Serono) was administered for ovarian stimulation. After 7 days of recombinant gonadotrophin therapy (300 IU daily for 2 days and then 75 IU daily for 5 days), sonography revealed the presence of a total of 22 follicles (15 of them measuring \( \geq 14\) mm in diameter and the five leading follicles measuring \( \geq 18\) mm) which were associated with serum oestradiol concentrations of 1864 pg/ml (endometrial thickness 12 mm). HCG (Profasi\textsuperscript{®}; Serono) from a different batch with respect to that used in the first treatment cycle was administered i.m. (5000 IU) on day 8 of stimulation and 36 h prior to transvaginal follicular puncture. Aspiration and repeated flushing of all follicles (\( n = 12\) ) in the right ovary failed to yield any identifiable oocyte. Follicular puncture was therefore suspended. The \( \beta\)-HCG and progesterone serum concentrations on the day of retrieval were 20 IU/l and 1.8 ng/ml respectively. A second dose of 5000 IU HCG from a different batch (the third one used in this patient after two treatment cycles) was given and a new oocyte retrieval was planned 36 h later. Again, no ova were obtained at this second attempt for oocyte retrieval despite the fact that both ovaries (follicles in the left ovary and four clear refilled follicular images in the right one) were punctured and aspirated. The \( \beta\)-HCG serum concentration was 48 IU/l and serum progesterone concentration was 7.8 ng/ml 36 h after the second dose of HCG.

A third treatment cycle was then planned using recombinant HCG (Ovidrel\textsuperscript{®}; Ares-Serono, Geneva, Switzerland) to induce ovulation, a drug which had not yet been marketed and that was generously provided by the manufacturer. The patient gave her informed consent to be treated with recombinant HCG and the study protocol was approved by the Spanish Health Authorities and the Ethics Committe of our hospital. In the third treated cycle, once pituitary–ovarian suppression was apparent, recombinant FSH (300 IU daily for 2 days and then 75 IU daily for 9 days) (Gonal-F\textsuperscript{®}; Serono) was used again for ovarian stimulation. After 11 days of ovarian stimulation, the oestradiol serum concentration was 1294 pg/ml in association with the presence of 12 follicles of which five were \( \geq 14\) mm in diameter and the two leading follicles were \( \geq 18\) mm. Endometrial thickness was 11 mm. A dose of 250 \( \mu \)g (5000 IU) of recombinant HCG (Ovidrel\textsuperscript{®}; Ares Serono) was then administered s.c. Follicular puncture and aspiration were performed 36 h later and five oocytes (three were metaphase II and two were prophase I) were retrieved. The \( \beta\)-HCG and progesterone serum concentrations 36 h after recombinant HCG administration were 61.8 IU/l and 4.7 ng/ml respectively. Three oocytes were injected with spermatozoa, three were fertilized, two cleaved, and two embryos were transferred. The luteal phase was supported with micronized vaginal progesterone. No pregnancy occurred.

Discussion
Since the original description (Coulam et al., 1986) in women with unexplained infertility, the lack of oocyte retrieval in women showing normal follicular development at ultrasound and with appropriate oestradiol response, has been seen by most IVF teams in patients having various infertility factors. A clear universal explanation of this event does not exist but different causes have been reported or postulated in the literature. In fact, EFS may be a multifactorial problem and synergism of aetiological factors to produce the syndrome in particular patients may exist. On the other hand, individual susceptibility to the genesis of the EFS seems clearly feasible considering that: (i) the syndrome is only seldom seen among groups of patients undergoing the same stimulation protocols and receiving the same batch of drugs; (ii) patients have different thresholds for follicular response to HCG (Abdalla et al., 1987); and (iii) there are patients in whom EFS tends to recur despite normal bioavailability of HCG and such cases have been attributed to a biological abnormality in the supply of mature oocytes that are retrievable (Awonuga et al., 1998).

The surrogate LH surge of administered HCG plays a crucial role in intrafollicular events that should have been completed before the time of oocyte retrieval, such as softening of the connective tissue elements of the follicle which facilitates the detachment of the oocyte–cumulus complex from the follicle wall (Meniru and Craft, 1997). Therefore, it is easy to understand those cases of EFS which occur, presumably due to the absence of an HCG injection (Asch et al., 1992; Quintans et al., 1998). On the other hand, the importance of the temporal relationship between onset (or administration) of the ovulation-inducing trigger and oocyte retrieval, in the pathogenesis of EFS, has been stressed in recent reports showing that if oocyte retrieval is attempted too early (only 12 h after HCG injection in the reported cases), a repeat aspiration can successfully be carried out at the more appropriate interval of about 36 h (Meniru and Craft, 1997; Quintans et al., 1998).

Apart from those human errors in the aetiology of EFS, it is now well accepted that decreased HCG availability, whatever its origin, seems to be a fundamental cause in many of the published cases of EFS (Zegers-Hochschild et al., 1995; Ndukwe et al., 1996, 1997; Ubaldi et al., 1997; Ali Hassan et al., 1998). Zegers-Hochschild et al. (1995) demonstrated that EFS was caused by rapid plasma clearance of the batch of HCG that was injected. This was attributed to some defect arising during the production, packaging or storage of the drug. Thus, the rapid clearance of the drug by the liver would prevent adequate exposure of relevant follicular processes to the action of HCG (Zegers-Hochschild et al., 1995). On this basis, treatment of EFS was initially proposed in those patients having \( \beta\)-HCG concentrations <10 IU/l 36 h after i.m. HCG.
injection when oocytes are not obtained from the follicles in one ovary despite aspiration and several flushes; the administration of a second ovulatory injection of HCG from a totally different batch yielded mature oocytes from the intact ovary, suggesting a fault with the HCG previously administered (Ndukwe et al., 1996, 1997). Recent reports, however, have shown that a possible ‘variant’ of the so-called EFS exists which is characterized by the salvage of the cycle after giving a second ovulatory dose of HCG and rescheduling oocyte retrieval for 24–36 h later, but only in patients having detectable immunoreactive HCG in serum and elevated progesterone serum concentrations on the day of the failed oocyte retrieval (Ubaldi et al., 1997; Ali Hassan et al., 1998; Awonuga et al., 1998). This contrasts with the absent immunoreactive HCG and the low serum progesterone concentration consistently reported by (Zegers-Hochschild et al., 1995) in their series. The threshold serum concentrations of HCG associated with pre-ovulatory changes within the follicle are not known for certain but on the basis of experimental work in rats and clinical data, it has been established that the minimum β-HCG concentration necessary for oocyte maturation in humans would be around 5–10 IU/l (Ndukwe et al., 1996, 1997). According to our experience, even β-HCG concentrations as low as 17 IU/l on the day of oocyte retrieval are associated with appropriate oocyte recovery and normal fertilization rate (unpublished observations). There is no definite explanation for this variant of the EFS which was clearly shown in our patient in the two first treatment cycles reported here but two possibilities have been raised. Either the dose of HCG injected had insufficient bioactivity or the ovaries showed an insufficient or delayed response to the administered dose (Ubaldi et al., 1997; Ali Hassan et al., 1998). Serum progesterone concentration was not investigated in the first treatment cycle in our patient but β-HCG concentration (46 IU/l) was almost identical to that observed in the third treated cycle (β-HCG = 48 IU/l) where progesterone concentration in serum had risen to 7.8 ng/ml 36 h after the second dose of HCG. This observation argues against the biological inactivity of the injected preparations.

Overall, data discussed above mainly favour the inappropriately low bioavailability of HCG as the crucial factor in the pathogenesis of EFS. In turn, this low bioavailability would be related to the drug itself, its administration or a very rapid metabolic clearance of the drug by the liver. A fact stressed to support the concept of EFS as a sporadic event related to HCG rather than a permanent pathological condition is that many reported cases of the syndrome had successful oocyte retrieval in earlier or later treatment cycles. The case reported here has several unique features in this regard. First, EFS was recurrent in the second ICSI attempt despite the use of HCG from a different batch with respect to the previous cycle. Second, the use of a second dose of HCG from a third batch during the second gonadotrophin stimulated cycle also failed to yield oocytes at the second retrieval. Thus, the EFS was recurrent despite the fact that the therapeutic approach currently proposed in the literature was applied. Finally, this is the first reported case of recurrent EFS ameliorated with the use of recombinant HCG.

Our patient had polycystic ovary syndrome and IVF in this condition has been reported to be often associated with a less favourable follicular micro-environment and an increased number of immature or unhealthy oocytes (Rosenwaks et al., 1996). It is accepted that the elevated LH concentration with the resulting abnormal follicular androgen milieu can exert an atretic effect on the growing follicle. Thus, a longer suppression prior to gonadotrophin stimulation seems necessary in polycystic ovary syndrome patients in order to neutralize the unfavourable intra-ovarian mechanisms that interfere with normal folliculogenesis. It could be postulated that an effect of pre-treatment with oral contraceptives favours the EFS but no previous report has suggested an association and the long GnRH agonist protocol with antecedent treatment with contraceptive pills seems an appropriate approach in those patients (Rosenwaks et al., 1996). Despite using this dual approach to gonadotroph and ovarian suppression in the three treated cycles in our patient, oocytes were obtained only when recombinant HCG was used to influence the final follicular maturation. It has been suggested that in patients showing an ovarian hyper-response (as frequently occurs with polycystic ovaries), the expression of LH receptors may be delayed or insufficient at the moment of HCG injection (Ubaldi et al., 1997). This possibility cannot be overlooked in the case reported here, considering that duration of ovarian stimulation was somewhat longer in the third treatment cycle. On the other hand, potential differences in the quality of urinary and recombinant HCG preparations or in their effects on the polycystic ovaries may be postulated.

The underlying mechanisms favouring recombinant HCG over urinary-derived HCG in this situation is a matter of speculation. However, like recombinant human FSH (Siebold, 1996), recombinant HCG is produced under the most stringent manufacturing conditions. Because the source of material (Chinese hamster ovary cell) is constant and the manufacturing process is quality assured, the resulting recombinant HCG is highly consistent from batch to batch. Recombinant HCG is the first gonadotrophin to be released in mass units, thus testifying to its high degree of consistency. Urinary HCG is still standardized and released by an in-vivo bioassay which is subject to inherent variability. Secondly, there may be present in urinary HCG enzymes co-purified during the manufacturing procedure, which may lead to desialyation of the molecule. In view of the published reports of variability in different batches of human menopausal gonadotrophin (Stone et al., 1989) and the reports of EFS, it is plausible to postulate that recombinant HCG may prove to be a more reliable ovulation inducing agent than urinary HCG. However, randomized clinical studies are required to support this contention.

References


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