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Empty follicle syndrome in two sisters with three cycles: Case report

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Empty follicle syndrome (EFS) is characterized by a lack of retrieved oocytes in the presence of multiple follicle development, in both natural and stimulated cycles. The aim of the present case report is to point out the possibility of genetic factors that could be responsible for some occurrences of EFS. Two sisters with moderate deafness underwent controlled ovarian hyperstimulation and IVF/ICSI cycles at the same centre. During all three cycles there were normal follicular development, estradiol levels and bio-available hCG plasma levels, but no oocytes and cumulus–corona complexes were retrieved, despite second hCG injections. These cases may represent an inherited condition of EFS with hearing loss with genetic factors affecting both the aetiology of EFS and the hearing loss.

Key words: cyclic AMP/empty follicle syndrome/ICSI/IVF

Introduction

Empty follicle syndrome (EFS) is a lack of retrieved oocytes from follicles at the time of repeated aspiration and flushing after ovulation induction, despite apparently normal follicular development and normal estradiol levels. EFS occurs in both natural and stimulated cycles (Zreik *et al*., 2000) with a reported incidence of between 0.6–7% (Awonuga *et al*., 1998), and is not a sporadic event. The pattern of ovarian response by ultrasonographic and hormonal follow-up is not helpful to predict EFS (Ben-Shlomo *et al*., 1991). The mechanism of EFS remains hypothetical. EFS might reflect dysfunctional folliculogenesis with early oocyte atresia and apparently normal hormonal response (Tsuiki *et al*., 1988). It has also been interpreted solely as a drug-related syndrome, resulting from an abnormality in the biological activity of some batches of hCG (Zeger-Hochschild *et al*., 1995; Ndukwe *et al*., 1997).

Congenital deafness (~70% non-syndromic, 30% syndromic), occurs in approximately one in 1000 live births and 50% of these cases are hereditary (85% of these cases have autosomal recessive transmission while 12–15% have an autosomal dominant form, X-linked forms represent 1–3%). There has been a tremendous progress in research on the genetic basis of deafness and mutations in 10 different genes for non-syndromic deafness have been identified since 1997. The GBJ2 gene encoding for gap junction protein connexin-26 (CX 26) has a high prevalence (one deaf person among 1765 people) and has been established as a common cause of autosomal recessive non-syndromic hearing loss. The identification of mutation in the GBJ2 gene should be a practical proposition for screening deafness (Lefebvre and Van De Water, 2000).

The aim of the present report is to highlight the possibility of genetic factors that could be responsible for some cases of EFS or even unexplained infertility. To the best of our knowledge, this is the first reported case of two sisters with sensorineural deafness having EFS in three IVF/ICSI cycles despite the rescue trial of follicles with second hCG injection.

Case report

Two sisters underwent controlled ovarian hyperstimulation (COH) and IVF cycles at the same centre and were followed by the same team. One of the sisters was 36 years old, with 17 years of primary infertility history. ICSI was planned for the couple after the diagnosis of unexplained infertility. On day 3 of spontaneous cycle, FSH and estradiol (E_2) levels were 8.8 mIU/ml and 68 pg/ml respectively. Pituitary desensitization was achieved by triptorelein (0.1 mg Decapeptyl®, Ferring, Germany) given s.c. from day 21 of the previous cycle. When serum E_2 level was $\geqslant$50 pg/ml, FSH stimulation was started as 300 IU per day, for 3 days. On day 3 of stimulation, when the E_2 level was 80.9 pg/ml, FSH was increased to 375 IU/day and continued in the same dose until hCG injection. E_2 levels were 286, 823, 1661 and 2358 pg/ml on days 5, 7, 9 and 10 of stimulation respectively. On the day of hCG injection, the levels for E_2, progesterone and LH were 3115 pg/ml, 1.8 ng/ml and 2 IU/ml respectively. The total number of follicles was 15 in both ovaries (4, $\geqslant$18 mm leading follicles in the right ovary;

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Table I. Follicular fluid hormonal profile in three cycles of ovarian stimulation

	1st cycle	2nd cycle	3rd cycle
Estradiol (ng/ml)	271	380	496
Testosterone (ng/ml)	8.4	9.3	6.5
Progesterone (ng/ml)	4942	3020	5168
LH (IU/ml)	1.1	2.2	0.29
FSH (IU/ml)	4.8	3.7	4.1
Inhibin B (pg/ml)	51	86	69
hCG (IU/ml)	22	13	461

5, ⩾18 mm leading follicles in the left ovary). hCG (10 000 IU) was administered for ovulation triggering on day 12 of stimulation and the E_2 level was 3335 pg/ml, 12 h post hCG. Follicular aspiration was performed transvaginally by ultrasound guided puncture, 35 h later. In the right ovary, neither oocytes nor cumulus–corona complexes were recovered after aspiration of seven follicles (4, ⩾18 mm) and aspiration was stopped following the diagnosis of EFS by the embryologist. We interrogated the patient and did not find out any erroneous administration of hCG. At that time, hCG plasma level was 262 IU/ml. A new dose of hCG (10 000 IU) was given for possibility of rescue despite bioavailability of hCG. Thirty five hours later, aspiration of the left ovary was attempted but neither oocytes, nor granulosa cells were recovered after aspiration of eight follicles (5, ⩾18 mm).

The patient's sister was 32 years old, with 14 years of primary infertility history. Two cycles of ICSI were tried with an interval of 3 months with a diagnosis of unexplained infertility; however both of the cycles resulted in EFS. During the first trial of ICSI, on day 2 of spontaneous cycle, FSH and E_2 levels were 7 mIU/ml and 60 pg/ml respectively. Leuprolide acetate (0.1 mg/day, s.c.) was initiated from day 21 of the cycle preceding COH and recombinant FSH (rFSH) (300 IU/day, i.m.) was started on day 3 of the subsequent cycle. E_2 levels were 149, 458 and 1152 pg/ml on days 4, 6 and 8 of stimulation respectively. On the day of hCG injection, the levels for E_2, progesterone and LH were 1722 pg/ml, 2 ng/ml and 1.2 IU/ml respectively. hCG (10 000 IU) was administered for ovulation triggering on day 10 of stimulation. Serum hCG and E_2 levels were 294 mIU/ml and 1626 pg/ml, 12 h post hCG. Total number of follicles was 10 in both ovaries (3, ⩾18 mm in the left ovary). When we aspirated three mature follicles from the right ovary, neither oocytes, nor cumulus–corona complexes were recovered and hCG level was 351 mIU/ml at that time. After careful interrogation of the patient we could not find any drug-related problem. Thirty-five hours after the second administration of hCG (10 000 IU) for possible rescue, we could not recover either oocytes or granulosa cells from the left ovary. A second cycle was performed after the first attempt. On day 2 of spontaneous cycle, FSH and E_2 levels were 6.3 mIU/ml and 20 pg/ml respectively. Pituitary desensitization was achieved by triptorelein (0.1 mg, Decapeptyl®, Ferring) given 0.05 mg s.c. from day 21 of the previous cycle and rFSH (450 IU day, s.c.) was started on day 3 of the subsequent cycle. E_2 levels were 388 and 1537 pg/ml on days 5 and 9 of stimulation respectively. hCG (10 000 IU) was administered on day 10 of stimulation with E_2, progesterone and LH levels of 1598 pg/ml, 0.9 ng/ml and 1.1 IU/ml respectively. E_2 level was 1357 pg/ml, 12 h post hCG and serum hCG level was 300 mIU/ml. Total number of follicles was 9 in both ovaries (4, ⩾18 mm leading follicles in the right ovary; 5, ⩾18 mm leading follicles in the left ovary). When follicular aspiration was performed from the left ovary, neither oocytes, nor granulosa cells were recovered again. hCG plasma level was 262 IU/ml, at that time. Despite detecting bioavailable hCG in plasma, hCG (10 000 IU) was administered for a second time. We did not recover any oocyte by aspiration of the right ovary, 24 h later. Follicular fluid levels of steroids, hCG, LH and inhibin B in three cycles are presented in Table I.

Interestingly, these two sisters have moderate hearing loss. Their parents are not relatives and the family history does not include hearing loss, infertility or any other abnormal finding. They have one other daughter who is unmarried. We completed the tests [including bilateral view head, cervical, thoracic, lumbar and vertebral X-rays, magnetic resonance imaging, audiologic examination and connexin 26 (GBJ2) gene study] and dysmorphic examinations for the diagnosis of syndromic–nonsyndromic hearing loss. After all these tests, the two sisters were diagnosed as having moderate sensorineural deafness. We found no GBJ2 mutations. Coding exon of connexin 26 gene was screened by SSCP method (Tekin *et al*., 2003).

Both couples had normal peripheral blood karyotypes and there was no evidence of hypogonadotrophic hypogonadism, history of anosmia, primary amenorrhoea and systemic disorders or neurological defect. During all three cycles, in the two sisters, there was normal follicular development, E_2 levels and bio-available hCG plasma levels. No oocytes and cumulus–corona complexes were retrieved, despite the second hCG injections, and subsequently no obtain cumulus–corona complexes were available for tissue culture.

Discussion

Ovulation is the end product of complex interactions of gonadotrophins and proteolytic cascade systems. Early oocyte atresia or strong attachment of oocyte–cumulus complex (Tsuki *et al*., 1988), altered folliculogenesis (Meniru and Craft, 1997) and drug-related syndrome leading to dysfunctional intrafollicular events (Zegers-Hochschild *et al*., 1995) are suggested as the aetiologies of EFS.

The surrogate LH surge of administered hCG has overall similar effects on follicular development. Poor quality of the drug, improper dosage or timing of hCG administration may result in an iatrogenic EFS. Ndukwe *et al*. (1997) reported their 'cure' for EFS and suggested a second attempt of collection after an extra bolus of hCG if the circulating levels of hCG were <10 IU/ml and oocytes were not retrieved from the first ovary. However, Awonuga *et al*. (1998) demonstrated that normal bioavailability of βhCG on the day of oocyte recovery does not exclude the diagnosis of EFS. Recent reports (Meniru and Craft, 1997; Hassan *et al*., 1998) suggested that some patients may need a longer exposure to hCG for the detachment of oocyte–cumulus complexes from the follicle wall. In our

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cases, the longer exposure to hCG was the rationale of waiting for 35 h before the second oocyte aspiration (rescue protocol).

In all three IVF cycles we aspirated the follicles from one ovary only and made a second attempt on the other ovary later, following a second hCG injection. The rationale for this approach was to increase the possibility of positive outcome with a second hCG injection. Considering IVF cycles as expensive and long-term therapies for the patients, we tried to increase the chances on the behalf of the patients in each cycle. Lack of oocytes and granulosa cells from the other ovary following second hCG injection confirmed our initial diagnosis of EFS.

EFS has been suggested to be a manifestation of ovarian dysfunction (Awonuga *et al*., 1998) and to represent an advanced stage of ovarian ageing (Ben-Shlomo *et al*., 1991; Zreik *et al*., 2000). Zreik *et al*. reported that there were no differences between the number of leading follicles of EFS and normal cycles in the same patient; however lower estradiol concentrations were observed on the day of hCG injection in EFS cases. Interestingly, this finding was also significant 2 days after the oocyte retrieval. They also found that no patient <34 years old had a recurrent EFS cycle. They suggested that the hampered granulosa cell function and/or metabolism led to altered oocyte growth and maturation and consequently to EFS.

In our cases with EFS, serum E_2, progesterone and LH levels on the day of hCG administration eliminate the possibility of early luteinization, premature ovulation and improper timing of hCG injection. Follicular fluid levels of steroids, hCG, LH and inhibin B in three cycles indicate that the follicles were neither atretic nor prematurely luteinized.

Overall infertility is not accepted as an inherited condition, however recent genetic studies have revealed that hereditary factors might be involved in the pathogenesis of subfertility and infertility. EFS in two sisters may result from either genetic origin or environmental factors; however hearing loss accompanied by EFS in two sisters with no history of hearing loss in other family members directed us to a genetic origin. To rule out the most frequent cause of non-syndromic hearing loss, we examined two sisters for the GBJ2 gene encoding connexin 26 and we found no mutations. To our knowledge, there are no data about inherited conditions of EFS and hereditary type of syndromic hearing loss associated with other system abnormalities. Perrault's syndrome, an autosomal recessive disorder, is associated with ovarian dysgenesis and sensorineural hearing loss. In some cases of Perrault's syndrome additional neurological findings (ataxia, nystagmus, sensory polyneuropathy) were reported (Cristiakos *et al*., 1969; Bozse *et al*., 1983); however the major finding was ovarian dysgenesis, hence our cases are not consistent with Perrault's syndrome. Another interesting case was reported by Lorda-Sanchez *et al.* consisting of sensorineural deafness, premature ovarian failure and chorioderma. Aetiology of this case was reported as balanced X-4 translocation where the breakpoint was within the critical region (Xq13–26) (Lorda-Sanchez *et al*., 2000). X-autosomal translocation causes disruption of the structural coding or regulatory sequences of the gene(s) or submicroscopic deletion of the region leading to a contiguous gene deletion syndrome. As described in this report, our cases could be a contiguous gene syndrome due to a deletion in a critical region or another region related to these symptoms. It can also be related to defect of a gene producing a protein that affect both granulosa cells and hearing function. As the parents are not relatives, autosomal dominant inheritance with reduced penetrance and/or expressivity, X-linked inheritance, or multifactorial inheritance are possible. Autosomal recessive inheritance can also be considered. 'A gene defect' in EFS may cause rapid dysfunction of granulosa cells leading to disordered periovulatory events. cAMP level is a potential modulator of granulosa cell function. Gonadotrophins regulate granulosa cell function primarily by altering the expression of cAMP-responsive genes involved in the regulation of cell proliferation, differentiation and apoptosis (Hillier *et al*., 1996). The underlying pathology of EFS may be related to post-receptor signalling system-cAMP that induces the expression of apoptosis genes too early, leading to rapid apoptosis before ovulation or deposition of an appropriate hyaluranic acid matrix for the free-floating of oocyte–cumulus cell mass. In our cases, molecular studies and clinical follow-up are necessary to establish premature ovarian failure. EFS is thought by some to be a drug-related phenomenon–pharmaceutical industry syndrome which is curable with a second hCG injection. In this paper, we concluded that EFS may result from a genetic disorder. Both EFS and moderate deafness may be part of a syndrome resulting from 'a single gene defect' or 'a contiguous gene syndrome' representing a new syndrome. These are the first EFS cases reported with moderate deafness. A number of similar cases, molecular studies and clinical follow-up are necessary in order to confirm or refute the hypothesis of a genetic aetiology of EFS.

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