The empty follicle syndrome (EFS) is defined as a lack of retrieved oocytes from follicles, at the time of repeated aspiration and flushing, following ovulation induction. The actual mechanism responsible for the EFS is still unknown. The aim of this study was to offer more information regarding the possible connection of this syndrome with pericentric inversion of chromosome 2. We give a case report of a patient who had multiple failed IVF attempts, due to the absence of oocyte and granulosa cells in the follicular fluid, following oocyte retrieval in both stimulated and natural cycles. Chromosomal analysis showed the presence of a pericentric inversion of chromosome 2: 46,XX.inv(2)(p11q21) in the female partner karyotype, while the male partner had a normal karyotype. Our case showed possible genetic factor influence in the aetiology of EFS.

Key words: chromosome 2/cytogenetic analysis/empty follicle syndrome/pericentric inversion

Introduction
Coulam et al. (1986) were the first to describe the empty follicle syndrome (EFS) 18 years ago. EFS is defined as a lack of retrieved oocytes from the follicles at the time of repeated aspiration and flushing following ovulation induction; it occurs despite apparently normal follicular development and serum estradiol levels. The incidence of EFS among patients undergoing IVF ranges from 0.2 to 7% (Ben-Shlomo et al., 1991; Awonuga et al., 1998; Quintans et al., 1998).

Several authors proposed that the cause of EFS might be dysfunctional folliculogenesis with early oocyte atresia, while patients show apparently normal hormonal response (Tsuiki et al., 1998). Others explain EFS in the light of a drug-related syndrome, resulting from an abnormal biological activity of some hCG batches (Zegers-Hochschild et al., 1995; Memiru and Craft, 1997; Ubaldi et al., 1997; Hassan et al., 1998; Papier et al., 2000). The actual mechanism responsible for EFS is still unknown. Onalan et al. (2003) described an empty follicle syndrome in two sisters with moderate deafness, and postulated the possibility of a genetic factor influence in the aetiology of EFS. A recent report from Lok et al. (2003) presented a successful treatment for EFS.

The aim of this study is to offer more information regarding this interesting issue, by presenting chronologically a case of a patient with EFS and pericentric inversion of chromosome 2. To our knowledge, this is the first described case of structural chromosomal rearrangement associated with this condition.

Materials and methods
A 32 year old patient was referred to our clinic 5 years ago (at the age of 27 years) for the assessment of secondary infertility. Her first pregnancy was spontaneous, at the age of 21 years, but ended spontaneously at 7 weeks of gestation. Following that pregnancy, she was unable to conceive for the next 6 years.

Transvaginal B-mode and colour Doppler ultrasound examination showed no pathology, nor did hysterosonosalpingography. On the 4th day of spontaneous cycle her serum hormone levels were FSH 8.1 IU/l, LH 4.1 IU/l, estradiol (E2) 118 pg/ml, prolactin 320 mIU/l. The patient and her husband underwent routine microbiological and molecular tests. Cervical and urethral swabs were tested for the presence of anaerobic and aerobic bacteria (by cultivation), Chlamydia trachomatis and Human Papilloma Viruses (by molecular tests DIGENE hybrid capture II, DNA, Digene Corporation, Gaithersburg, Maryland, USA, according to manufacturer’s instructions).

Husband’s semen analysis demonstrated asthenozoospermia (3 ml volume; total of $48 \times 10^6$ motile sperm; $7 \times 10^6$/ml of rapid progressive, $9 \times 10^6$/ml of slow progressive, $2 \times 10^6$/ml of non-progressive, and $24 \times 10^6$/ml of immotile sperm respectively), which is slightly aberrant from normal sperm count and does not compromise fertility. Other close family members did not have fertility problems; the patient had five sisters and four brothers and they all had...
at least three children. Her family history was negative regarding hereditary disorders or consanguinity.

The couple was diagnosed with unexplained infertility. We subsequently tried several assisted reproduction procedures, which are summarized in Table I. The medications used for intrauterine inseminations (IUI) were clomiphene citrate (CC) (Klomifen; Belupo, Koprivnica, Croatia), recombinant (r)FSH (Gonal F; Serono, Geneva, Switzerland) and hCG (Primogonyl; Schering AG, Berlin, Germany). Following three unsuccessful IUI cycles, we attempted IVF in two consecutive spontaneous cycles. The hormone values on day 2 of the first spontaneous cycle were: E₂ 176 pg/ml, FSH 5.5 IU/l, LH 4.5 IU/l. Serum inhibin B on day 3 of the cycle was 163 pg/ml. It was measured in duplicate by two-side enzyme-linked immunosorbent assay (ELISA) (Oxford Bio-Innovation Inhibin-B Immunoassay Kit, Serotec Ltd, UK) using a monoclonal antibody raised against the inhibin βA subunit in combination with a labelled antibody raised against the inhibin α subunit as previously described (Groome et al., 1996). Transvaginal ultrasound on day 12 showed an 11 mm follicle and 6 mm thick hyperechoic endometrium. On day 18, E₂ was 809 pg/ml. Hormonal and ultrasonography findings in the second spontaneous cycle attempt were very similar. In each cycle, the ovulation was triggered with hCG and follicular aspiration was performed 36 h after that.

We proceeded with a down-regulation treatment protocol. The medications used were oral contraceptive pills (OCP) that contained 0.03 mg of ethinyl estradiol and 0.15 mg of levonorgestrel (Stediril M; Krka&Wyeth, Novo Mesto, Slovenia), GnRH analogue buserelin (Suprefact; Hoechst, Frankfurt, Germany), hMG (Pergonal; Serono, Geneva, Switzerland), and hCG. Details on drug administration are shown in Table I. We were unable to find oocytes or granulosa cells in the follicular fluid.

Following that unsuccessful attempt, she became pregnant spontaneously in the next cycle. Cytogenetic examination of aborted material showed a female fetal karyotype with pericentric inversion 2q21. The fragile sites are breakpoint locations in the pericentromeric region and 2q21 region that is described as a fragile site. The fragile sites are also the possibility of gene structure disruption within the region, producing the effect of partial monosomy. There is a dosage-sensitive gene effect is caused by X-autosomal translocations with breakpoint sites downstream from XIST site (Xq13.2) producing an effect of disomy for that segment. Some of the genes located on autosomal segment of translocation can be inactivated under the regulation of XIST region, producing the effect of partial monosomy. There is also the possibility of gene structure disruption within the breakpoint sites. Dysfunctional folliculogenesis can be caused by alternations of expression of involved genes.

A gene mutation in one of the still unknown genes included in pathogenesis of EFS may cause rapid dysfunction of granulosa cells leading to disordered periovulatory events. Our patient had pericentric inversion of chromosome 2 with breakpoint locations in the pericentromeric region and 2q21 region that is described as a fragile site. The fragile sites are places of increased genetic instability and their expression is greatly influenced by both genetic and environmental factors.

### Discussion

The genetic mechanisms of EFS are still unknown. Few cases with EFS and associated abnormalities of other organ systems have been reported. Perrault’s syndrome, an autosomal recessive disorder, associated with ovarian dysgenesis and sensorineural hearing loss, was not consistent with our case.

Lorda-Sanchez et al. (2000) reported another interesting patient with sensorineural deafness, premature ovarian failure and choriorderma. A balanced translocation involving chromosomes 4 and X was found, with the breakpoint site Xq21.1 within the critical region for premature ovarian failure. A dosage-sensitive gene effect is caused by X-autosomal translocations with breakpoint sites downstream from XIST region, producing the effect of partial monosomy. There is also the possibility of gene structure disruption within the breakpoint sites. Dysfunctional folliculogenesis can be caused by alternations of expression of involved genes.

A gene mutation in one of the still unknown genes included in pathogenesis of EFS may cause rapid dysfunction of granulosa cells leading to disordered periovulatory events. Our patient had pericentric inversion of chromosome 2 with breakpoint locations in the pericentromeric region and 2q21 region that is described as a fragile site. The fragile sites are places of increased genetic instability and their expression is greatly influenced by both genetic and environmental factors.
Furthermore, the presence of pericentric inversion could cause large disturbances in the pairing and alignment on the meiotic spindle, influencing the normal process of folliculogenesis.

The timing of the early rise in inhibin B concentration in plasma suggests that antral follicles secrete inhibin B in response to FSH. However, it is unclear whether the inhibin B originates from one selected dominant follicle or from all the antral follicles. Our patient did not have polycystic ovaries on ultrasound and therefore this would not explain the higher concentration of inhibin B found early in the cycle. She also had normal FSH values. FSH stimulates the secretion of inhibin from granulosa cells and, in turn, is suppressed by inhibin—a reciprocal relationship. Inhibin B is involved in regulatory functions in developing follicles and appears to be a sensitive marker of ovarian follicle development, as well as an important inhibitor of FSH secretion. Secretion of dimeric inhibin B, therefore, may be dependent upon a FSH-induced increase in the level of α-subunit expression to reach the relative excess of α-subunit mRNA required for dimeric inhibin synthesis (Mason et al., 1987).

Groome et al. (1996) found that inhibin B plasma concentration was high in the early follicular phase (86.8 ± 13.8 pg/ml) and fell in the late follicular phase. We found day 3 inhibin B level in our patient to be above the normal range (162 pg/ml), with FSH levels in the normal range. Low inhibin B values in the follicular phase are usually consistent with high FSH values and are thought to be an early sign of

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**Figure 1.** Partial karyotype 2: 46,XX,inv(2)(p11q21). Green arrows:. Black arrows:. LHCGR - luteinizing hormone/choiogonadotropin receptor FSHR - follicle-stimulating hormone receptor INHBB - inhibin, beta B ACVR1 - activin A receptor, type I INHA - inhibin, alpha ⇧ - breakpoint site
oocyte pool depletion (Clausen et al., 1995; Rubov et al., 2000; Tinkanen et al., 2001; Morreti and Toscano, 2003; Loverro et al., 2003; Meden-Vrtovec, 2004). On the other hand, higher inhibin B values in the follicular phase have not been discussed thoroughly in the literature. Nevertheless, inhibin B gene is found on the same chromosome involved in our structural rearrangement, and mutations of inhibin B gene may be responsible for premature ovarian failure (Shelling et al., 2000). Several genes on chromosome 2 are involved in fine regulation of the menstrual cycle. The order of genes in the inversed segment is reversed. It is possible that chromosomal rearrangements have direct positional effects (i.e. the establishment of rearrangements might induce changes in the expression patterns of associated genes). Indeed, experimental evidence suggests that expression patterns can be altered around the breakpoints of chromosomal rearrangements (Marques-Bonet et al., 2004; Srebniak et al., 2004).

Different IVF treatment methods could also modulate the response and the successful oocyte recovery in EFS cases, therefore we tried different treatment modalities, albeit unsuccessfully. Onalan et al. (2003) proposed that cases with EFS should be clinically evaluated for the presence of additional congenital anomalies such as sensorineural deafness. If such anomalies are present also, they could be the part of some contiguous gene syndrome due to a deletion in a critical region or another region related to these symptoms. Further molecular and clinical studies should be performed in order to obtain more information about the genetic basis of EFS.

References


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