# Distribution of etiological factors of hypergonadotropic amenorrhea

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**Summary**

*Objective:* To describe the etiology of hypergonadotropic amenorrhea (HA) and outline the subgroup of infertile women that might achieve pregnancy with their own eggs despite premature ovarian failure. *Methods:* In this retrospective study we enrolled 70 women aged 32.5 ± 5.71 years. After a detailed history of the disease, measurements of follicle-stimulating hormone, estradiol, prolactin and thyroid-stimulating hormone levels, determination of the karyotype and fragile X premutation syndrome, and a quick ACTH test, estrogen-progestin replacement therapy was introduced. *Results:* In 17 of the 70 women, HA was due to chromosomal abnormalities, in 16 to extensive gynecologic surgery, in ten to oral contraceptive use, in four to chemo- and radiotherapy; in 23 HA was idiopathic. After estrogen-progestin replacement therapy, three women with idiopathic HA conceived and delivered healthy babies. *Conclusion:* Estrogen-progestin replacement therapy in pharmacological doses might be beneficial to women with idiopathic HA, having normal prolactin levels, adrenal and thyroid function, and a normal karyotype.

*Key words:* Estrogen-progestin treatment; Hypergonadotropic amenorrhea; Pregnancy.

## Introduction

Etiologies of hypergonadotropic amenorrhea (HA) in the reproductive period are diverse; if HA occurs before the age of 40, it is defined as premature ovarian failure (POF). The diagnosis of POF is made in around 10% of patients complaining of secondary amenorrhea [1].

The terminology used to categorize patients with POF has led to considerable confusion regarding etiologic factors. Friedman *et al.* [2] proposed a classification of primary ovarian failure. The initial division is between women with an intact number of immature follicles (gonadotropin-resistant ovarian syndrome) and women with premature follicular depletion. Those with follicular depletion are further divided regarding their chromosomal complement. Chromosomally abnormal women represent a complete unit, whereas normal subjects with 46 XX karyotype are divided into those having familial, immunologic, iatrogenic, infectious or secondary to systemic diseases or idiopathic etiology.

Therapeutic efforts to restore ovarian function have been focused on 46 XX subjects with follicular depletion and on those with gonadotropin-resistant ovary syndrome [3]; as for chromosomally abnormal subjects, there have been anecdotic reports of accidental pregnancies achieved with estrogen treatment [4].

The aim of this study was to describe the etiology of hypergonadotropic amenorrhea (HA) in women referred to our outpatient infertility clinic. Our further aim was to identify a group of women without any known or proven reason for HA (idiopathic HA), and within this group a subgroup of those that could achieve pregnancy with their own eggs.

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## Materials and Methods

In this observational uncontrolled retrospective study we enrolled 70 hypergonadotropic women seeking infertility treatment in the outpatient clinic of the Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, in the time period 2002-2007. To find etiologic factors of amenorrhea, the women underwent determinations of the levels of follicular stimulating hormone (FSH), estradiol ($E_2$), prolactin and thyroid-stimulating hormone (TSH), and karyotyping and fragile X premutation analysis.

In all women serum FSH and E2 levels were checked twice in a time lapse of two of three months.

Determinations of the analyzed hormones were performed on the fully automated analyzer LIAISON, Dia Sorin, Saluggia, Italy) with a quantitative direct competitive chemiluminescence immunoassay (CLIA). Each test is a modified two-step (sandwich principle) assay where a specific antibody to the measured hormone is bound to magnetic particles.

Normal values for follicular phase FSH range between 3.5 and 9.2 IU/l, for $E_2$ between 0.17 and 0.41 nmol/l, for prolactin between 6.2 and 23.5 μg/l, and for TSH between 0.3 and 3.6 mU/l.

Karyotyping and the analysis of the fragile X premutation were performed according to standard procedures [5].

In all the women vaginal ultrasound (US) examination was repeated two to three times by an independent observer to assess antral follicle count and to exclude or confirm ovarian resistance syndrome. In all the enrolled women no follicles were registered; the endometrial thickness was < 4 mm.

Since the $E_2$ level in all women was far below the normal range and US endometrial thickness was less than 4 mm, a progesterone withdrawal test was not performed.

The mean women's age was 32.5 ± 5.71 years (range: 19-40 years), and the duration of amenorrhea ranged from 1-15 years. Prior to enrollment in the study and the confirmed diagnosis, the women had been receiving either continuous or discontinuous HRT regimen or oral contraceptives.

After a close examination of the history data, complete hormonal determinations, karyotyping and fragile X premutation

analysis, and after a confirmed diagnosis of HA, estrogen-progestin replacement therapy consisting of a daily dose of estradiol valerate 2 mg and norgestrel 0.5 mg was introduced.

The study was approved by the national medical ethics committee and each woman signed an informed consent before entering the study.

## Results

The women's history data showed that among the 70 enrolled women 16 had undergone extensive pelvic or ovarian surgery, ten had used oral contraceptives (OC) for more than two years prior to onset of amenorrhea, and four had had chemo- and radiotherapy for malignant disease.

Karyotyping revealed that 17 women had chromosomal abnormalities.

Table 1. — *Presumed etiology of hypergonadotropic amenorrhea.*

| Etiology | Diagnosis (n) | n (%) |
|---|---|---|
| Extensive surgery | Endometriosis (8)<br>Viscerolysis OWR (8) | 16 (22.9) |
| OC | > 2 years (10) | 10 (14.3) |
| Radio-, chemotherapy | Hodgkin's disease (3)<br>Leukemia (1) | 4 (5.7) |
| Genetic | 45XO (7)<br>46XY (2)<br>Mosaicism (8) | 17 (24.3) |
| Total | | 47 (67.2) |

OC = oral contraception; OWR = ovarian wedge resection.

On the basis of the women's history data and karyotyping, etiologic factors were established in 47 of the 70 women (67.1%). In the remaining 23 women (32.8%) no history data of toxic, infectious, medicamentous, endocrine or environmental risk factors could be identified; these women were therefore classified as having idiopathic HA. After estrogen-progestin replacement therapy, three of the 23 women (13.3%) with idiopathic HA conceived and delivered healthy babies.

In the three women that conceived FSH levels before pregnancy were found elevated and estrogen levels decreased. Thyroid function assessed by TSH determination was found to be normal in all three patients, and so were prolactin plasma concentrations (Table 2).

Table 2. — *Clinical data on women who conceived.*

| Patient initials | Age (years) | Duration of amenorrhea | FSH (IU/l) | $E_2$ (nmol/l) | PRL (µg/l) | TSH (mU/l) |
|---|---|---|---|---|---|---|
| HDB | 35 | 1 year | 109.98 | 0.04 | 19 | 0.5 |
| KKD | 21 | 1.5 years | 93. 88 | 0.07 | 16 | 2.1 |
| KS | 35 | 1 year | 73. 69 | 0.09 | 12 | 0.9 |

$E_2$ = estradiol, FSH = follicle-stimulating hormone, PRL = prolactin, TSH = thyroid-stimulating hormone.

During pregnancy no hormonal support was offered, and the three women delivered healthy babies; one of the women had twins.

Karyotypes of the women that conceived were normal; also, these women had no history of any risk for the development of HA. One conceived while waiting for oocyte donation, and two during the time period when deciding either on oocyte donation program or adoption.

Table 3. — *Clinical data on babies.*

| Mother (initials) | Baby's sex | Birth weight | Apgar score | Pediatric estimation |
|---|---|---|---|---|
| HDB | Male | 3,020 g | 9 | Normal |
| (twins) | Male | 2,990 g | 8 | Normal |
| KKD | Male | 3,770 g | 9 | Normal |
| KS | Female | 3,450 g | 9 | Normal |

Ten to 12 months after delivery FSH levels were measured in the three women again demonstrating hypergonadotropic FSH (HDB: 90.5 and 92.9 IU/l; KKD: 49.6 and 65.8 IU/l; KS: 62.1 and 71.2 IU/l).

## Discussion

Beyond ovum donation there has been no proven effective therapy in patients with HA to achieve pregnancy. The etiology of the disorder is heterogeneous and by selecting the patients according to the known causative factors, a group of those with possible fertility perspectives could be identified. The aim of this study was to describe the etiology of HA and identify the subgroup of infertile women that could benefit from estrogen-progestin replacement therapy, and define their characteristics.

According to Powell *et al.* [6] chromosomal abnormalities are detected in 40-50% of women with HA. In our group we registered only 24% of chromosomal abnormalities, which is likely due to the selected group of women seeking infertility treatment. Women with chromosomal abnormalities (monosomy X, mosaicism, polysomy X) may have ovarian function preserved at least for a limited period of time [4]. In these women early diagnosis is of outmost importance as it offers a possibility of cryopreservation of ovarian tissue for future fertility [7].

Several reports have identified the women with HA among fragile X premutation carriers with a familial history of premature ovarian failure (POF) [8]. Sherman, using the combined information from women interviewed at the age of ≥ 40 years, estimated the rate of POF among fragile X premutation carriers to be 21% [9]. The frequency of fragile X premutation carriers among women with sporadic POF has been found to range between 1.6 and 3.3% [8, 10]. In Slovene women with sporadic POF, evaluated at the Department of Obstetrics and Gynecology Ljubljana between 1991 and 2001, the fragile X premutation was found in 4.8% of the screened women (4/83 women) [5].

No case of fragile X premutation was registered among the women enrolled in this study.

Iatrogenic damage of ovarian tissue encompasses cytotoxic treatment used in leukemia and Hodgkin's disease, and extensive ovarian or pelvic surgery. Long term use of OC may hide the signs of ovarian insufficiency and post-

pone the possibility of early diagnosis and treatment. Cytotoxic-induced ovarian failure is notable for occasional spontaneous remission, although approximately one half of all women receiving 400-500 rads to ovaries over four to six weeks will develop permanent ovarian failure [11, 12].

A case report by Menashe *et al.* [13] of a patient who conceived spontaneously on three occasions resulting in a live-birth infant is a proof that gonadal failure following chemotherapy may not be permanent.

In our group of women with HA, three had undergone treatment for Hodgkin's disease and one for leukemia. In these women the hypergonadotropic condition persisted for more than ten years, which renders the possibilities of spontaneous remission and reverse of ovarian function most unlikely.

Extensive pelvic surgery with direct ovarian damage or resection may lead to premature depletion of ovarian follicles, ovarian cortical damage, and hence a functional loss in the ovarian reserve [14].

We registered 16 cases of HA after surgery; in eight HA occurred after extensive surgery of pelvic and ovarian endometriosis, and in eight after extensive viscerolysis with ovarian wedge resection. We speculate that surgical procedures were too aggressive to preserve the ovarian function. In cases of removed or destroyed ovarian tissue no treatment for infertility can be considered, therefore these women are offered hormonal replacement therapy, oocyte donation or adoption.

Following OC use, women often experience some delay in resuming normal menses, but according to most studies [15] less than 1% fail to begin menstruating regularly within six months. About one-half of these will develop post pill amenorrhea resulting from a disruption of the normal hypothalamic-pituitary-ovarian function. In evaluating the women with post pill amenorrhea it is important to rule out hyperprolactinemia, weight changes and POF. The medical history of women with post pill amenorrhea should be thoroughly analyzed focusing on menstrual cycle regularity before OC treatment is prescribed. In our series of HA women, ten had used OC for more than two years. According to the data from the literature there appears to be no correlation between the duration of OC use and the occurrence of amenorrhea [15].

Women with menstrual cycle irregularities should be checked for the basic hormone levels, i.e. FSH, LH, PRL and TSH, before they are prescribed OC. The ten OC users in our series had had irregular menstrual cycles and had been prescribed OC without searching for the cause of irregularities: six had been prescribed OC to abolish menstrual irregularities, and four for the prevention of unwanted pregnancy. This situation clearly indicates the importance of considering the indications and contraindications prior to OC prescription.

Between 10% and 30% of women with HA have a concurrent autoimmune disease, the most common being hypothyroidism. In all our women the thyroid function was assessed, and the results were normal. HA has also been reported to be associated with myasthenia gravis, systemic lupus erythematosus, rheumatoid arthritis and Chron's disease [16].

Another autoimmune disorder that affects ovarian function is Addison's disease. We previously reported a case of a woman with Addison's disease that developed POF and hypothyroidism [17]. After estrogen-gestagen replacement therapy, and treatment with thyroid hormones and corticosteroids, she conceived and delivered a healthy baby. Additionally, autoimmune lymphocytic oophoritis was also reported in association with adrenal insufficiency (Addison's disease) as steroidogenic cell immunity [16].

In a large proportion of women the cause of HA is not identifiable. It seems likely that occult viral oophoritis might account for many of these cases [18].

In their systematic review on therapeutic interventions to restore ovarian function, Van Kasteren and Schoemaker [19] evaluated 52 case reports, eight observational studies, nine uncontrolled studies and seven controlled trials. Due to a strong variability in study design, patient selection and type of intervention, it was not possible to combine the data of seven controlled studies to perform a meta-analysis. According to their conclusions there was no evidence that any treatment could enhance pregnancy rate. However, anecdotal reports of pregnancies that occurred in women with HA during treatment with estrogens or estrogens and gestagens [20-22] provide evidence for a possible recruitment of residual oocytes should they exist.

Achievement of three pregnancies in our series of women with idiopathic HA after estrogen-progestin treatment is in accordance with the statements that estrogen or estrogen and progestin treatment may be beneficial in some types of HA. Differentiation among different types of HA is essential when deciding on the introduction of therapeutic interventions. At present a promising predictor of ovarian reserve is anti Mullerian hormone level determination exceeding conventional analytes testing with FSH and inhibin B. In the future it could become a screening test to detect the women with residual oocytes.

In the era of assisted reproduction which covers nearly all cases of severe infertility, infertility due to HA can be overcome with an oocyte donation program. A great majority of women wishing pregnancy enter these procedures, but there are still some who wish to have their own genetic child. Therefore, it seems reasonable to identify those women with HA that may benefit from hormonal treatment. Based on our results these are the ones with idiopathic HA, normal prolactin levels and adrenal and thyroid function, with normal karyotype, and with no history of extensive pelvic surgery.

Young women with HA should be offered higher doses of estrogen replacement therapy than women in their fifties. Not only to cope with vasomotor symptoms but also to increase target tissue responsiveness by increasing the concentration of its own receptor and that of the intracellular progestin and androgen receptor [23]. In addition, the efficacy of estrogen treatment depends also on the time of initiation of therapy. Our results are in agreement

with the results of Lin and Yu [24], who, based on 126 HA cases, found a significantly better efficacy of estrogen treatment among the cases with amenorrhea lasting less than one year as compared to those lasting longer than one year.

In conclusion, we have to emphasize that women with HA who wish to conceive with their own eggs should be properly investigated of the etiology of the disease. A selected group of women, apparently those with idiopathic HA that have no extensive pelvic surgery in their history, and have normal prolactin levels, thyroid and adrenal functions, and a normal karyotype, should be offered immediate estrogen-gestagen treatment in pharmacological doses together with detailed and objective counseling.

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