Reply to Reviewer #1

Dear Reviewer,

Thank you very much for reviewing our manuscript and your very encouraging comments.

Reviewer Comments:
a) The work is in general well written and in general easy to follow. The work is also highly detailed, well conducted, and well organized. The clinical history of the three patients is well detailed.
b) The majority of references in the field are covered, while papers adequately described and critically discussed in the context of study findings. The scientific writing is good. Figures are highly explicative and clear.
c) Figures and tables are highly explicative and clear.

We also appreciate your clear and detailed feedback and hope that the explanation we provide below will fully address your concerns. In the remainder of this letter, we discuss each of your comments individually along with our corresponding responses.

To facilitate this discussion, we first retype your comments in italic font and then present our responses to your comments.

Comment 1:
d) *More details on the Intermittent spontaneous ovulation should be included as background. Authors can check PMID: 26291617 and PMID: 30357029*

Response 1:
Thank you for these valuable comments. We have checked the literature carefully and added more details relating to intermittent spontaneous ovulation into the INTRODUCTION part of the revised manuscript (Line 47-52, Line 339-345).

Comment 2:
e) The work should be carefully revised for the presence of typos.

Response 2:
Thank you for this comment. The revised manuscript has been edited by the Charlesworth Group, as recommended by the Editor-in-Chief.

Comment 3:
f) Line 165 follicular maturity disorders also included infections (PMID:34970247 and PMID: 33556108), this notion should be included

alongside supporting references

Response 3:
As suggested, we have added two references to support our idea relating to follicular maturity disorders (Line 354-361).

Comment 4:
g) Line 167 The global menopause mean age should be included

Response 4:
Thank you for your suggestion to improve the accessibility of our manuscript. We have now added the mean age of global menopause (Line 180).

Comment 5:
h) Lines 200-203 these sentences would better fit at the beginning of the discussion

Response 5:
Thank you for this comment. We have re-written this part according to the Reviewer's suggestion and moved the sentences mentioned to the beginning of the discussion (Line 160-163).

Thank you for your comments and suggestions. We hope that you find our manuscript much improved. The revised areas are shown in yellow highlight for your convenience. We look forward to hearing from you in due course.

Yours sincerely,

Wanyu Zhang

Intermittent spontaneous ovulation in patients with premature ovarian failure: three case reports

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Keywords: Premature ovarian failure; Follicular development; Natural pregnancy; Artificial cycle; Case report

Abstract

Premature ovarian failure (POF) is the end-stage of a decline in ovarian function prior to the age of 40 years that involves symptoms associated with low estradiol (E_2) levels and a minimal probability of pregnancy. This increases the physical and psychological burden experienced by young women of reproductive age, particularly with regards to over-diagnosis. Here, we report three cases (29, 22, and 33 years-of-age) diagnosed with POF after experiencing secondary amenorrhea for more than one year, serum levels of follicle-stimulating hormone (FSH) >40 IU/L on two occasions with an interval of more than 4 weeks, and negative progesterone withdrawal tests. All three patients were intermittently administered with drugs to create an artificial cycle. During the subsequent discontinuation period, the patients experienced intermittent follicular growth and spontaneous ovulation. One patient experienced two natural pregnancies (both with embryo arrest). Our findings suggest that young patients with POF can experience unpredictable and intermittent spontaneous follicular development, ovulation, and even natural pregnancy. Clinicians should provide appropriate medical guidance and individualized treatments according to fertility requirements, genetic risks and hypoestrogenic symptoms as soon as possible.

Keywords: Premature ovarian failure; Follicular development; Natural pregnancy; Artificial cycle; Case report

Introduction

Premature ovarian failure (POF) refers to ovarian failure prior to the age of 40 years and represents a heterogeneous gynecological and endocrine disease with multiple causes. The clinical manifestations of POF are amenorrhea, high levels of gonadotropin (Gn), and symptoms associated with low estradiol (E_2) levels, including hot flushes, sweating, vaginal dryness, and infertility.[1] The global incidence of POF is 1–5% and affects approximately 2 million women of reproductive age.[2] The known causes of POF include genetic, immune, infection, metabolic, environmental, and psychological factors; enzyme deficiencies, and iatrogenic injury. However, 50–90% of patients experience POF with an unknown etiology which is referred to as idiopathic POF.[3] Unlike physiological menopause, which occurs within a predictable age range, approximately one-third of women with secondary POF have dormant follicles.[4] Furthermore, under stimulation by high levels of FSH, patients can experience intermittent follicular development, E_2 secretion, and even ovulation. Intermittent spontaneous ovulation is known to occur in women with both regular (> 33%) and irregular menstruation; this can increase the risk of osteoporosis and cerebrovascular disease.[5] Compared with primary amenorrhea, POF patients with secondary amenorrhea are more likely to experience intermittent spontaneous ovulation; this can be predicted by the analysis of FSH, E_2 and inhibin B levels rather than the levels of

anti-Mullerian hormone (AMH).[6]

In this article, we summarize the diagnosis and treatment of three cases of POF experiencing intermittent spontaneous ovulation who were admitted to Peking Union Medical College Hospital (PUMCH) between December 2012 and July 2022. We also review the relevant literature and discuss the characteristics, possible causes, diagnosis, and treatment options for each case.

Case data

Case 1

This patient was delivered by cesarean section at full term from her mother's first pregnancy in 1983. The pregnancy was uneventful with no history of special medication. The patient showed no differences when compared to her peers with regards to growth and intelligence. Menarche occurred at 13 years-of-age followed by irregular menstruation (7/20–180 days). The patient married in 2010 (at 27 years-of-age) and did not use birth control after marriage. The patient experienced normal menstruation on the 27th of November 2011 followed by amenorrhea. On the 4th of December 2012 (at 29 years-of-age), she attended PUMCH with the chief complaint of secondary amenorrhea for one year. FSH was 110 IU/L, E_2 was < 15 pg/ml, and thyroid function was negative. After 41 days, we re-analyzed hormonal activity: FSH was 120 IU/L, and E_2 was < 15 pg/ml. Chromosomal status was 46, XX. Pelvic ultrasound showed that the uterus was normal, and no follicles were detected in the ovaries. There was no previous history of mumps, surgery, or chemoradiotherapy, and no special family history. Following a negative progesterone withdrawal test, the patient was diagnosed with POF and administered with medicine (Climen) to generate an intermittent artificial cycle. She stopped taking the drug after menstruation on the 6th of January 2016. After having sex only once on the 21st of January 2016, examinations revealed that the level of human chorionic gonadotropin (HCG) was 884 IU/L on the 6th of February 2016 due to breast swelling and pain. However, embryo arrest occurred at 9 weeks. Uterine evacuation was performed and chorionic villus sampling revealed a chromosomal status of 46, XN. Menstrual relapse occurred 35 days after surgery followed by occasional natural menstruation and monophasic basal body temperature. If menopause lasted more than three months, the patient applied the artificial cycle treatment by herself, recording FSH fluctuations of 55–110 IU/L. In 2018, the patient received donor egg preimplantation genetic testing for aneuploidy (PGT-A) in Taiwan. Two freeze-thaw blastocysts were transplanted, and the patient successfully conceived two pregnancies. In September 2019, a baby boy and a baby girl were delivered by cesarean section. The patient continued postpartum breastfeeding for two months. At six months postpartum (March 2020), the patient experienced a natural menstrual relapse and then continued to menopause. In May 2020, FSH was > 40 IU/L and E_2 < 15 pg/ml. In July 2020, a natural pregnancy was detected again. Unfortunately, uterine evacuation was performed due to embryo arrest without analysis of the chorionic villus. On the 26th of October 2020, FSH was 101.44 IU/L, luteinizing hormone (LH) was 50.29 IU/L, and E_2 was < 15 pg/ml without hot flushes, and sweating. Progestogen was administered intermittently from December 2020 to October 2021 before being changed to an artificial cycle

(Femoston (2/10)) (Fig. 1); the patient remains on this treatment.

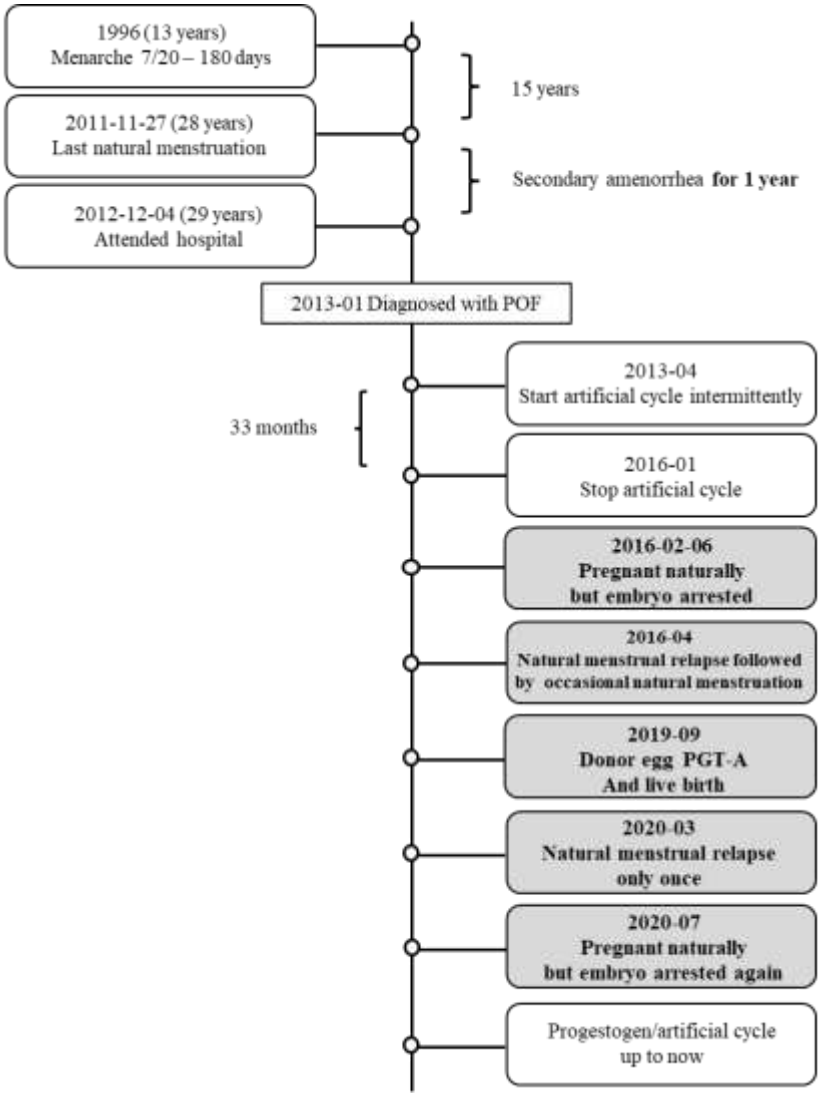

Fig. 1. The timeline of case 1

Case 2

This patient was born from her mother's first pregnancy. The pregnancy was uneventful with no history of special medication; delivery occurred spontaneously in 1998. Growth and intelligence did not differ significantly from her peers. Menarche occurred at 14 years-of-age (4–5/30–60 days; medium volume). On the 7th of December 2019, she underwent normal menstruation but then experienced postmenstrual amenorrhea (at 21 years-of-age). She was unmarried and had no sex. She attended PUMCH with the chief complaint of secondary amenorrhea for more than one year on the 11th of February 2021; FSH was 54.75 IU/L, LH was 5.32 IU/L, E_2 was 26.01 pg/ml, progesterone (P) was 0.3 ng/ml, testosterone (T) was 0.71 ng/ml, prolactin (PRL) was 10.3 ng/ml, and AMH was 0.03 ng/ml. After 44 days, we re-analyzed hormonal activity: FSH was 72.30 IU/L, LH was 39.58 IU/L, E_2 was 26.45 pg/ml, P was 0.42 ng/ml, and T was 0.45 ng/ml; thyroid function was negative. Ultrasound showed that the uterus and both ovaries were small. Chromosomal status was 46, XX and the fragile X syndrome test was normal. The patient denied hot flushes and sweating. She had no previous history of mumps, surgery,

radiotherapy, chemotherapy, and no special family history. After a negative progesterone withdrawal test, the patient was diagnosed with POF. Following a menopause hormone therapy (MHT) safety review, she began an artificial cycle (Femoston (2/10)) in April 2021. During the period of drug administration, she experienced regular menstrual cycles (4–5/28–30 days) without discomfort. On the 19th of September 2022, the patient stopped taking the drug by herself. On the 12th of October 2022, she experienced natural menstruation, lasting for 4 days, and a hormonal review showed that FSH was 20.70 IU/L, LH was 4.34 IU/L, E_2 was <15 pg/ml, P was 0.81 ng/ml and T was 0.47 ng/ml. Natural menstruation was experienced on the 21st of November 2022 followed by repeat episode of amenorrhea. After more than 2 months, she recommenced the artificial cycle; the patient remains on this course of treatment (Fig. 2).

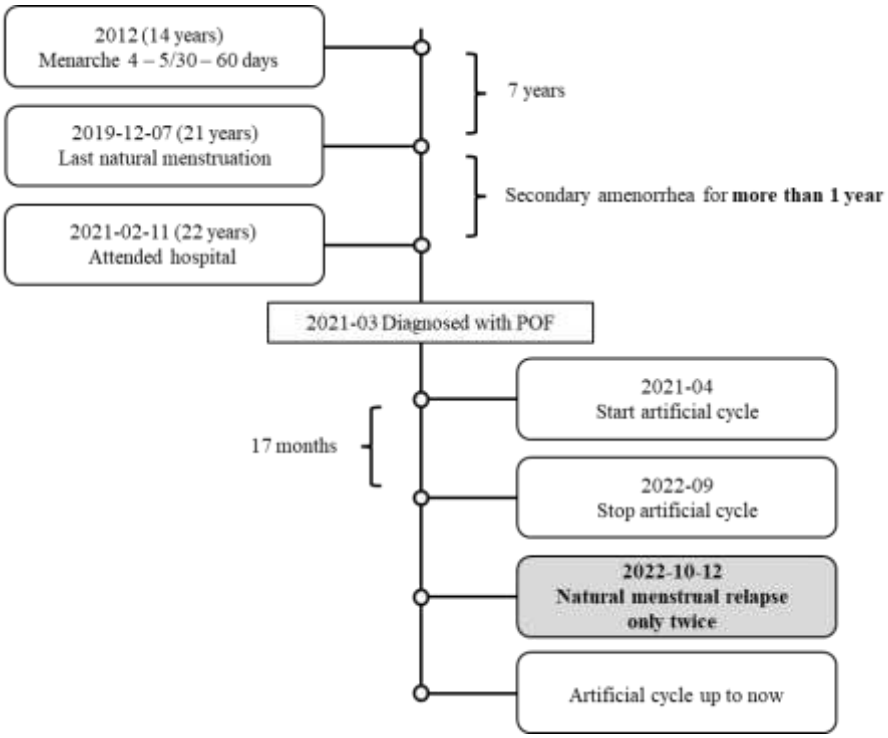

Fig. 2. The timeline of case 2

Case 3

This patient was born from her mother's second pregnancy. Her mother had an uneventful pregnancy with no history of special medication and delivered spontaneously in 1989. Growth and intelligence are no different from peers. Menarche occurred at 13 years-of-age (4/24 – 25 days, medium volume). At 24 years-of-age (2013), she was married and experienced a natural pregnancy in 2014. However, she experienced embryonic arrest after 2 months of pregnancy and underwent uterine evacuation without chromosomal analysis. In 2016, she became pregnant naturally, and underwent a second-term induction of labor in the fifth month of pregnancy due to social factors (G_2P_0). Subsequently, she was divorced. On the 27th of May 2021, she experienced normal menstruation followed by amenorrhea. On the 29th of June 2022 (33 years-of-age), she attended PUMCH with the chief complaint of secondary amenorrhea for more than one year, accompanied by hot flushes and sweating.

Hormonal status was as follows: FSH was 44.90 IU/L, LH was 20.65 IU/L, E_2 was 8.15 pg/ml, P was 0.51 ng/ml, T was 0.37ng/ml, PRL was 18.92 ng/ml, and AMH was 0.16 ng/ml. On the 31st of July 2022, FSH was 162.47 IU/L, LH was 106.80 IU/L, E_2 was < 15 pg/ml, P was 0.62 ng/ml, and T was 0.13ng/ml; thyroid function was negative. The patient's chromosomal status was 46, XX. The fragile X syndrome test was normal. Transvaginal ultrasound showed that the uterus and both ovaries were small. The patient had no history of mumps, surgery, radiotherapy, or chemotherapy, no special family history, and there were no abnormalities of menstruation and childbirth with regards to the patient's mother and sister. The patient was subsequently diagnosed with POF. Following a MHT safety inspection, carried out in August 2022, the patient began an artificial cycle (Femoston (2/10)). After one month of medication, the symptoms of hot flushes and sweating were significantly relieved. In December 2022, the patient changed the artificial cycle to traditional Chinese medicine (TCM) by herself. From February 2023, the patient experienced natural regular menstruations (3–4/28–30 days) without hot flushes and sweating (Fig. 3); the patient's status remains the same.

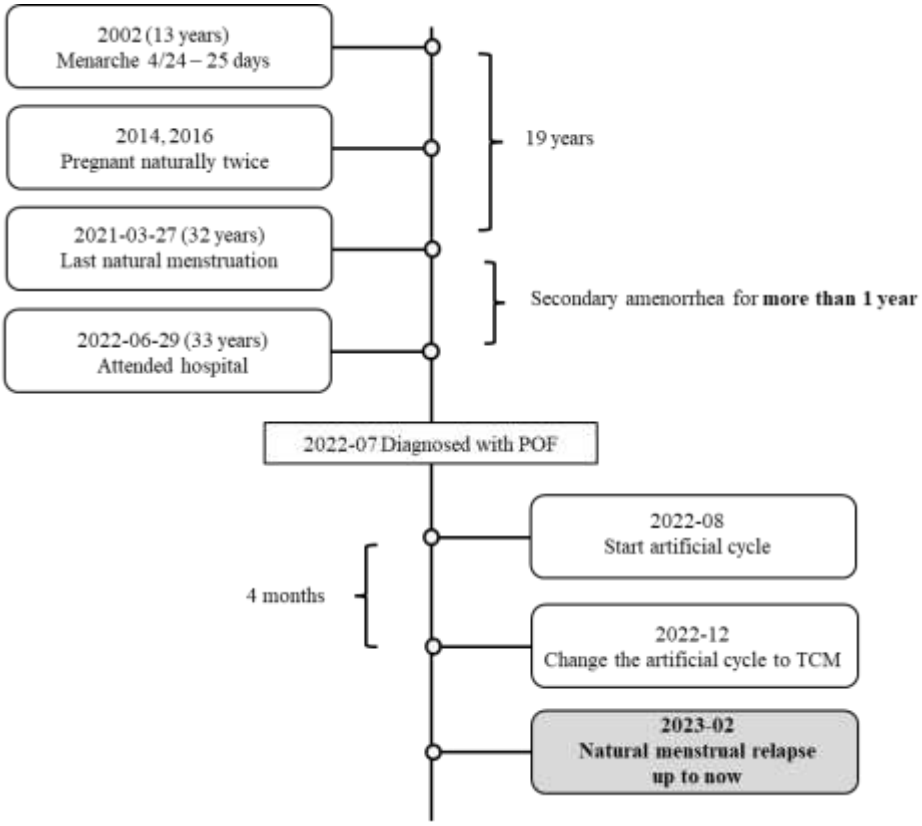

Fig. 3. The timeline of case 3

Discussion

In the present study, we describe three patients who were diagnosed with POF following negative progesterone withdrawal tests at the ages of 29, 22, and 33 years due to secondary amenorrhea for more than 1 year, and serum FSH levels > 40 IU/L on two occasions with an interval of more than 4 weeks.

Follicular development and POF

Human follicles begin to develop and reach a maximal number during the fetal period.

Subsequently, the number of follicles gradually decreases; there are 6–7 million follicles in the ovaries at 20–28 weeks-of-gestation, up to 3 million at birth, and 300,000–400,000 at puberty.[7] Follicular development is divided into two stages: initial recruitment and cyclic recruitment. Initial recruitment is the process of transition from the primitive follicle pool to a primary follicle until the germ stem cells and primordial follicles are fully depleted; this process is independent of Gn. During the monthly menstrual cycle after puberty, the follicles that have initiated recruitment respond to the periodic changes in Gn and then enter cyclic recruitment.[8] Therefore, the duration of natural menstruation (the period during which the ovaries fulfill their physiological role) is not only related to the original follicle pool reserve, but also depends on the original follicular recruitment rate and the rate of follicular atresia, including accelerated follicular atresia, dominant follicle recruitment abnormalities [9] and follicular maturity disorders,[10, 11] which may lead to POF.

Globally, the average age at which natural menopause occurs is 48.8 years;[12] approximately 90% of women aged 45–55 years are in menopause. Menopause that occurs between 40 and 45 years-of-age is referred to as early menopause. Ovarian failure before the age of 40 years is referred to as POF and involves a natural state or pathological state that results in the acceleration of follicle utilization. When the number of original follicles is lower than a certain threshold, the natural state no longer activates development or results in extremely slow development; even high levels of FSH are unable to induce the recruitment of follicles.

Diagnostic criteria for POF

In 1967, Moraes-Ruehsen and Jones [13] were the first to define POF as an abnormal physiological menopause after puberty and before the age of 40 years. Subsequent research has shown that ovarian failure is a gradual process. In 2006, the American Society for Reproductive Medicine (ASRM) first proposed the term primary ovarian insufficiency (POI).[14] In 2016, the European Society of Human Reproduction and Embryology (ESHRE)[15] and the International Menopause Society (IMS)[16] changed this definition to premature ovarian insufficiency (POI) and clarified the difference between POI and POF by dividing ovarian failure into three stages: the occult phase, the biochemical abnormality phase, and the clinical abnormality phase. Further division included diminished ovarian reserve (DOR), POI, and POF, according to the levels of FSH. The diagnostic criteria for POF are as follows: [1] amenorrhea for more than 1 year before the age of 40 years; [2] FSH > 40 IU/L on two occasions with an interval of more than 4 weeks, and [3] decreasing E_2 levels.[17] Significantly, some patients only have a history of amenorrhea that is less than one year when they first attend hospital; some of these patients commence artificial cycles at once. Therefore, it is difficult to distinguish POI from POF.

In addition, POF needs to be differentiated from resistant ovary syndrome (ROS). Patients with ROS present with amenorrhea, high levels of gonadotropin, and low levels of E_2; these conditions are often accompanied by infertility. These symptoms are similar to those of POF. However, ROS ovaries contain a large number of primitive follicles, and multiple small follicles can be detected in the ovaries under ultrasound.

The pathogenesis of ROS may be related to Gn receptor deficiency, the presence of antibodies that affect receptor activity, structural abnormalities of Gn molecules, or thymic lesions.[18]

POF may cause intermittent spontaneous ovulation

Although POF is the terminal stage of POI, ovarian function is not necessarily completely lost. The pathophysiology of POF differs from that of menopause in terms of average age. The follicles in the ovaries of menopausal women at a normal menopause age are almost completely exhausted; this condition is permanent. Women with POF are young, and some patients may have a certain number of follicles in a resting state in their ovaries that may be activated under certain conditions.[19]

There are two pathological types of POF.[20] [1]. Type I (with no follicles) involves the complete depletion of follicles due to the absence or inability of germinal cells to develop. This condition is often secondary to disorders of sex development. [2] Type II (with follicles) involves the presence of follicles in the ovaries in a resting state. The younger the patient is, the greater the possibility of being induced or spontaneously restoring follicle development. Type II POF follicles may gradually deplete and develop into type I follicles. The three patients described in this study were all young (29, 22, and 33 years of age) and used artificial cycles for several months. Natural menstruations were experienced after stopping the drug, thus indicating intermittent follicle recovery and development. We hypothesize that all three cases were type II POF, and that follicles in the resting state intermittently resumed growth when driven by high levels of FSH.

In addition to this, natural follicle development in women with POF may also be associated with MHT. Previous research has confirmed that the proliferation and differentiation of granulosa cells (GCs) depend on the levels of FSH, LH, PRL, and their membrane receptors. However, when GCs are exposed to a high Gn state for an extended period of time, the number and sensitivity of their receptors decreases (13, 14), thus causing low Gn sensitivity in the residual follicles in the ovarian tissue of patients with POF. In MHT, the levels of E_2 is independent of the number, distribution, and affinity of FSH receptors on GCs, but can promote the formation of FSH receptors and improve the sensitivity of FSH receptors on GCs by reducing the levels of FSH, thereby promoting follicle development and maturation. Moreover, levels of LH in the serum of patients with POF continues to rise, thus hindering spontaneous ovulation and the premature luteinization of follicles.[21] MHT can improve the ovulation rate by reducing the levels of LH and by reducing the inappropriate luteinization of follicles. Collectively, these factors make it possible for a very small number of patients with POF to undergo natural menstrual relapse.

The existing literature only features a small body of research relating to spontaneous ovulation and natural pregnancy in POF patients. There are several factors that might be related to this lack of research. For example, a non-standardized diagnosis of POF, such as a duration of amenorrhea for less than 1 year, an inappropriate number of FSH tests, or variable diagnostic thresholds.[22-24] Another factor could be the small number of POI and POF cases in which ovulation and pregnancy are under medical intervention,

such as the MHT process,[25, 26] ovulation induction (OI),[27] controlled ovarian stimulation (COS),[28] donor egg *in vitro* fertilization and embryo transfer (IVF-ET),[29] *in vitro* activation (IVA) (30), or ovarian tissue cryopreservation and auto-transplantation.[31]

In this study, all three POF patients intermittently resumed spontaneous ovulation when they stopped MHT treatment. One of the patients experienced two spontaneous pregnancies. These data suggest that clinicians should not ignore the intermittent and unpredictable restoration of follicle development in POF patients, and remember that there is still a possibility of unintended pregnancy during intermittent MHT treatment. If there is no reproductive plan, contraception should be recommended. Furthermore, if there are clear genetic factors (e.g., Turner's syndrome, fragile X syndrome, and pseudohypoparathyroidism), patients should be fully informed of the risks of pregnancy, ovarian dysfunction in the offspring, and other disorders.

Treatment strategies of POF

At present, there is no effective means of restoring POF ovarian function. Therefore, women with POF should be detected as early as possible so that they can be diagnosed and treated early to alleviate low E_2 symptoms, reduce long-term risks and protect residual fertility.

In terms of hormone supplementation, primary POF manifests as undeveloped or delayed female secondary sex characteristics. Secondary POF can cause hot flushes, sweating, osteoporosis, mood swings, cognitive decline, cardiovascular and cerebrovascular symptoms, dryness of the genital tract and dyspareunia (27) which cause adverse effects on the physical and mental health of patients. MHT can effectively alleviate the low levels of E_2 and long-term risk of POF patients and improve a patient's quality-of-life. However, MHT has a small risk of breast cancer and thrombosis. Thus, annual safety checks are required before and during medication. In the present study, one of our three patients experienced hot flushes and sweating symptoms (3 times/day). After safety examination, MHT was given, and the symptoms were significantly relieved after 1 month of medication.

In terms of fertility, methods for POF patients include the transplantation of previously frozen ovarian tissue, oocyte activation, stem cell therapy, dehydroepiandrosterone, growth hormone, antioxidants, immunosuppressants and egg donation. However, there is no effective treatment to reverse ovarian function.

In addition, TCM has a certain effect on the treatment of POF in younger patients. TCM considers that female reproduction is regulated by the brain-kidney-chong ren-uterine axis which is similar to the hypothalamic-pituitary-ovarian (HPO) axis in modern medicine. TCM could be used to supplement the kidney, replenish vital energy, and activate the blood.[32] In the present study, one of our POF patients experienced natural menstruation after changing artificial cycle to TCM. This patient has maintained regular natural menstruation thus far, therefore indicating that TCM may be beneficial to younger patients with POF, although multicenter, large-sample, and randomized controlled studies are still needed in future.

Conclusion

POF patients experience several symptoms, including amenorrhea, high levels of Gn, low E_2 levels and infertility. Data from the three patients described herein suggest that clinicians should adhere strictly to the diagnostic criteria for POF patients (< 40 years-of-age, amenorrhea for more than 1 year and serum FSH >40 IU/L on two occasions with an interval > 4 weeks), reduce the psychological pressure caused by over-diagnosis among young women, and inform women with POF that there is an intermittent and unpredictable possibility of resuming ovulation. Clinicians should also provide appropriate medical guidance according to whether there are fertility requirements and genetic risks. Furthermore, clinicians should commence individualized treatments as soon as possible, including hormone supplementation and fertility preservation.

Conflict of Interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Wanyu Zhang reviewed literature and drafted the manuscript. Hanbi Wang obtained patient consent. Chengyan Deng revised the manuscript.

Funding

This research was supported by a grant from the National High Level Hospital Clinical Research Funding (reference number: 2022-PUMCH-B-080, 2022-PUMCH-C-064).

Acknowledgments

The authors would like to thank the three cases for supporting our research.

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