Idiopathic premature ovarian failure: what is the most suitable ovarian stimulation protocol?

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Summary

Objective: To evaluate the ovarian response to ovarian stimulation in women with idiopathic premature ovarian failure (POF) in a prospective, controlled, and sequential crossover pilot study. *Materials and Methods:* Ten women with idiopathic premature ovarian failure and normal karyotype were included in the study. Phase I was comprised of three consecutive control cycles consisting each of estrogen progestin sequential therapy. Phase II was comprised of three consecutive treatment cycles combining the use of gonadotropin-releasing hormone agonist (GnRHa) in the background of estrogen priming, followed by gonadotropin ovarian stimulation and corticosteroid immunosuppression. *Results:* Ovulation rates in the treatment cycles (0/10; 0%) did not differ from control cycles (0/10; 0%). *Conclusions:* The findings of this pilot study showed that the combination of estrogen priming, corticosteroid immune-suppression, GnRHa pituitary desensitization, and followed by gonadotropin ovarian stimulation is ineffective in restoring ovarian function in women with idiopathic POF.

Key words: Premature ovarian failure; Ovarian stimulation; Gonadotropins; Corticosteroids; Gonadotropin-releasing hormone agonist; Estrogen replacement.

Introduction

Premature ovarian failure (POF) is the loss of normal ovarian function before the age of 40 years. While affected women suffer mostly from amenorrhea and climacteric symptoms, infertility remains the most significant concern. Although oocyte donation is considered the treatment of choice, it is an unacceptable option for many women and societies. Many forms of follicular stimulation protocols have been suggested to enhance reproductive outcome, but remain highly controversial. Such treatments include estrogen replacement therapy for ovarian sensitization [1, 2], gonadotropin-releasing hormone agonists (GnRHa) for pituitary down-regulation [3-5], corticosteroid administration for immune suppression [6-10], and exogenous gonadotropins for ovarian stimulation. Research on the effect of such therapies among women with POF is limited. In addition, results are often flawed by the fact that women have the potential to ovulate on their own even if they are not on therapy.

The aim of this pilot study was to examine the clinical benefits of a treatment protocol designed to address all hypotheses proposed for ovulation induction in POF (estrogen priming, GnRHa pituitary desensitization, corticosteroid immunosuppression, and gonadotropin ovarian stimulation) on the improvement of ovarian function in women with the idiopathic spontaneous type of this disorder.

Revised manuscript accepted for publication August 7, 2012

Materials and Methods

Patient population

The authors approached a sample of thirty-one women with idiopathic POF with an age range of 18-35 years who presented to a hospital-affiliated private fertility clinic seeking pregnancy. All refused oocyte donation and were offered to be enrolled in the clinical trial, which extended over a period of three years. This study was approved by the Institutional Review Board. All patients were informed about the investigational nature of the treatment, its uncertain outcome, and related psychological consequence; only ten (32.3%) agreed to take part in the trial and signed informed consents.

Women were diagnosed with POF if they had a baseline serum follicle-stimulating hormone (FSH) levels > 40 mIU/ml and estradiol (E2) levels < 20 pg/ml obtained on two separate instances with clinical amenorrhea exceeding six months. Inclusion criteria were: age range between 18 and 35 years, normal 46 XX karyotype, normal autoimmune profile (antinuclear antibodies, thyroglobulin antibodies, thyroid microsomal antibodies, antiperoxidase antibodies), normal hysterosalpingographic evaluation, and normal semen parameters. Excluded were patients who received chemotherapy or radiotherapy.

Study protocol

Three months prior to the trial, all hormonal medications were stopped. The clinical trial which consisted of two phases had a prospective, controlled, and sequential crossover design.

Phase I: Women received cyclical hormonal replacement therapy for three consecutive cycles: conjugated oral estrogens and daily continuously (1.25 mg) and oral medroxyprogesterone acetate daily for 12 days cyclically (10 mg).

Phase II: Women underwent three consecutive treatment cycles. Pituitary desensitization using the GnRHa buserelin acetate (600 μg intra-nasally daily) was started seven days prior

to the end of the 12-day progestin administration period. Corticosteroid immunosuppression therapy (40 mg orally daily) was also initiated at the same time. Both medications were continued until the end of each treatment cycle in parallel with the daily dose of conjugated estrogens. Controlled ovarian stimulation using human menopausal gonadotropins (HMG) (225 IU intramuscularly daily) was started on the second day of menstruation and for at least ten days thereafter. Each treatment cycle was preceded by a washout cycle during which cyclical hormonal replacement therapy was administered as described above in control cycles.

Transvaginal ultrasound (TVUS) examinations were performed at baseline and at five-day intervals. Follicular development was considered significant if the follicle largest diameter was 12 mm or above, time at which HMG therapy was continued beyond ten days. Human chorionic gonadotropins (hCG) (10,000 IU single intramuscular injection) were proposed when a follicle exceeding 16 mm in diameter was observed.

Serum E2 measurements were obtained on baseline and at five-day intervals. A rising serum E2 level ≥ 50 pg/ml was considered as a marker of ovarian steroidogenic activity. Serum progesterone (P) was obtained after seven days of hCG administration, and a serum level of ≥ 3.0 ng/ml was considered as an indicator of ovulation. Pregnancy was confirmed by a positive serum β-hCG titer.

Measures

The primary clinical outcome measures were the occurrence of ovulation (follicle largest diameter > 16 mm and serum luteal P level of ≥ 3.0 ng/ml). The secondary clinical outcome measures included: follicular development (follicle largest diameter ≥ 12 mm), ovarian steroidogenic activity (rising serum E2 level ≥ 50 pg/ml), and successful pregnancy (serum β-hCG titer ≥ 5 mIU/ml).

Statistical analysis

The Fisher exact and chi-squared tests were utilized for data analyses. In order to confirm the success of the proposed treatment protocol over expectant management, a 20% increase in ovulation rate was to be expected. Power analysis showed that 60 women were needed to detect the proposed difference in ovulation rate ($\alpha = 0.05$; $\beta = 0.1$; one-tailed test), assuming the initial findings of the pilot study are promising.

Results

A preliminary analysis of collected data on the first ten treated women demonstrated the failure of the proposed treatment protocol to achieve ovulation in any woman over twenty-five treatment cycles in Phase II and thirty control cycles in Phase I.

Women's characteristics are shown in Table 1. The mean age was 26 years (range, 18 - 32). All women were nulliparous and only three had primary amenorrhea (30%). The mean duration of amenorrhea among women with secondary POF was 21 months (range, six - 36). None of the patients had menstruation during the previous six months. One woman had a positive family history of POF, and none had a history of autoimmune disorders. All women underwent a total of three control cycles in phase I, but only five completed the third treatment cycle in phase II (an attrition rate of 50%).

Table 2 shows the stimulation characteristics of two women found to have active ovarian steroidogenesis in response to the treatment protocol (patients 7 and 8). Ultrasound examinations in the sample population showed the presence of small-sized follicles in six women (20%) on baseline at the start of Phase I and in four (16%) at the onset of Phase II. Detected baseline follicles underwent progressive development beyond 12 mm in diameter in two (6.7%) control and one (4.0%) treatment cycle. None however matured beyond 16 mm to meet the criteria for hCG administration. Limited steroidogenic activity was also detected in all three cycles (Table 3). No women had ovulation and consequently none got pregnant.

Discussion

The findings indicate that the use of the combination treatment protocol described, administered in the dosage and for the duration proposed, did not enhance ovarian and/or reproductive functions in estrogen-primed women with idiopathic spontaneous POF.

The hypothesis that POF causes an irreversible ovarian process was rejected by many investigators who reported a 5% to 10% spontaneous conception rates following initial diagnosis [11]. This fact led several researches to seek the use of medical therapies to improve the follicular response in affected women [1, 5-7] with many controversial findings [2-4, 8, 12, 13]. The present study differs from all previously reported ones in that it evaluated a treatment approach that combined all the therapies previously described in the literature in a single protocol. Estrogen supplementation is believed to enhance follicular activity in an estrogen-depleted system by increasing gonadotropin receptor sensitivity [14]. While Tartagni *et al*. [13], using a double-blind randomized placebo-controlled design, showed that pre-treatment with estrogens significantly improved successful ovulation rates (32%) in women with POF, Taylor *et al*. [1], using a randomized controlled cross-over design failed to demonstrate similar effects. In an attempt to suppress a presumed putative gonadotropin-driven antigen, Check *et al*. [2] in an uncontrolled prospective study, demonstrated successful ovulations and pregnancies, following GnRH-a pituitary suppression prior to gonadotropin ovarian stimulation. These findings were not supported by Nelson *et al*. [3]. As an autoimmune oophoritis was suggested in women with POF [11]. Badawy *et al*. [15] found a significant improvement in ovulation rates (20.7%) when dexamethasone was randomly added to GnRHa plus gonadotropin therapy in women with idiopathic POF. These findings however could not be confirmed by another randomized placebo-controlled study [8].

Because of the protean nature of POF and its many etiologies, a combination treatment approach addressing multiple biological hypotheses, was considered reasonable. Such a strategy was adopted previously by two groups of investigators who reported beneficial results in women with POF [12, 15]. Blumenfeld *et al*. [12]

Table 1. — *Individual characteristics of women with idiopathic POF.*

Patient no.	Age (y)	Parity	Type of amenorrhea	Duration of amenorrhea (mo)	Family history of POF	No. of control cycles	No. of treatment cycles
1	30	0	Secondary	24	Negative	3	2
2	22	0	Primary	–	Negative	3	3
3	27	0	Secondary	18	Negative	3	3
4	18	0	Primary	–	Negative	3	3
5	27	0	Secondary	18	Negative	3	2
6	24	0	Primary	–	Negative	3	3
7	24	0	Secondary	6	Negative	3	2
8	27	0	Secondary	12	Negative	3	2
9	32	0	Secondary	36	Positive	3	2
10	30	0	Secondary	30	Negative	3	3

Table 2. — *The stimulation characteristics of two women with idiopathic POF who had evidence of ovarian follicle-like activity..*

Patient	Phase	Cycles order	Baseline follicles	Follicular development	Steroidogenic activity	Ovulation	Pregnancy
7	I	3	+/+/+	-/-/+	-/-/+	-/-/-	-/-/-
	II	2	+/+	+/-	+/-	-/-	-/-
8	I	3	+/+/+	-/-/+	-/-/+	-/-/-	-/-/-
	II	2	+/+	-/-	-/-	-/-	-/-

(+) indicates a positive finding; (-) indicates the absence of any positive finding.

Table 3. — *The stimulation characteristics of two women with idiopathic POF who had evidence of ovarian follicle-like activity.*

Patient	Baseline follicles (N)	Developing follicles (N)	Follicle maximal diameter (mm)	Maximal estradiol level (pg/ml)
Study phase I				
7	2	1	14	65
8	1	1	12	72
Study phase II				
7	2	1	14	90

demonstrated high ovulation (87%) and conception (40%) rates with corticosteroid therapy and GnRH a pituitary suppression followed by HMG ovarian stimulation in POF of autoimmune origin over three consecutive treatment cycles. Similarly, Badawy *et al*. [15] found a significant improvement in ovulation (20.7%) and conception (6.9%) rates in women with idiopathic karyotypically normal POF utilizing a similar combination approach. The present findings failed to demonstrate any measurable benefit in terms of follicle development, ovulation or conception rates in women with idiopathic spontaneous POF treated with the proposed combination stimulation protocol. Despite minor differences in the types, dosages, and time duration of the different agents utilized in these three studies, the most plausible explanation for differences in clinical response rates observed, remains linked to the protean nature of the ovarian disorder and to variations in population genetics and ethnic characteristics.

Ultrasonography was proposed as a non-invasive alternative to assess follicular reserve, and was reported to depict ovarian follicle-like structures in 41% to 60% of patients with POF [16]. Mehta *et al*. [16] demonstrated that the presence of ovarian follicles on baseline vaginal ultrasonography was correlated with a better response to ovulation induction therapy. Such an observation, however, was not supported by other investigators [17]. Nelson *et al*. [17] found a poor correlation between serum E2 concentration and follicular diameter, and concluded that baseline follicles seen on ultrasonography are more likely to be dysfunctional in nature. The present findings concur with the latter view. All three cycles that expressed some follicular activity on stimulation as evidenced by ultrasound and biochemical findings, had originally small follicle-like structures on baseline ultrasound examination prior to initiation of therapy. However, in none did this activity lead to advanced folliculogenesis and ovulation. It follows that the presence of baseline ovarian follicles on ultrasound examination in women with POF is probably of little predictive value and is unlikely to yield any useful follicle response to ovarian stimulation.

One of the major limitations of this study is that a high proportion of women with POF initially approached declined enrollment into the study, yielding a low response rate of 32.3%. This has significantly narrowed the general applicability of these results. In addition, the high "drop-out" rate after the second treatment cycle (Phase II) resulted in an incomplete data set. It also showed that the only two women who experienced positive ovarian follicle-like activity during Phase II belonged to this group, which introduced bias by depriving the study from a number of treatment-response observations in a potentially more responsive group of women. The authors also employed conjugated estrogens for supplementation, which unlike ethinyl-E2, express some cross-reactivity with the serum 17-β E2 assay, potentially interfering with the interpretation of serum E2 data.

In conclusion, estrogen-primed women with idiopathic spontaneous POF did not appear to benefit from a multidisciplinary approach that combined pituitary down-regu-

lation, immune suppression, and gonadotropin ovarian stimulation. Infertile women with POF who reject oocyte donation and seek ovarian stimulation, need to be counseled extensively a priori on the low response rates of any investigational treatment strategy proposed. Justice is made by enhancing patient autonomy, which is best-served by explicitly explaining the doubtful beneficence of such investigational treatment strategies.

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