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Serum anti-Müllerian hormone and ovarian morphology assessed by magnetic resonance imaging in response to acupuncture and exercise in women with polycystic ovary syndrome: secondary analyses of a randomized controlled trial

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Key words
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Conflict of interest
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Abstract

Objective. To investigate whether electro-acupuncture or physical exercise influence serum anti-Müllerian hormone (AMH), antral follicle count (AFC) or ovarian volume in women with polycystic ovary syndrome (PCOS). *Design.* Secondary analyses of a prospective, randomized controlled clinical trial. *Setting.* University Hospital, Sweden. *Patients.* Seventy-four women with PCOS recruited from the general population. *Interventions.* Women with PCOS were randomized to 16 weeks of electro-acupuncture (14 treatments), exercise (at least three times/week), or no intervention. *Main outcome measures.* Serum AMH recorded at baseline, after 16 weeks of intervention, and at follow up at 32 weeks. AFC, and ovarian volume assessed by magnetic resonance imaging at baseline and at follow up at 32 weeks. *Results.* After 16 weeks of intervention, serum levels of AMH were significantly decreased in the electro-acupuncture group by 17.5% ($p < 0.001$), and differed from the change in the exercise group. AMH remained decreased by 15% ($p = 0.004$) also at follow up at 32 weeks, but did not differ from the exercise or the no intervention groups. There was a decrease by 8.5% ($p = 0.015$) in ovarian volume between baseline and follow up in the electro-acupuncture group, and by 11.7% ($p = 0.01$) in AFC in the physical exercise group. No other variables were affected. *Conclusions.* This study is the first to demonstrate that acupuncture reduces serum AMH levels and ovarian volume. Physical exercise did not influence circulating AMH or ovarian volume. Despite a within-group decrease in AFC, exercise did not lead to a between-group difference.

Abbreviations: AFC, antral follicle count; AMH, anti-Müllerian hormone; EA, electro-acupuncture; MRI, magnetic resonance imaging; PCO, polycystic ovaries; PCOS, polycystic ovary syndrome; RCT, randomized controlled trial.

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder associated with hyperandrogenism, oligo-anovulation, and polycystic ovaries (PCO); at least two of these characteristics are required for the diagnosis (1). Anti-Müllerian hormone (AMH) is a peptide produced by the granulosa cells of predominantly pre-antral and small antral follicles (2). Measurement of serum AMH is emerging as a potential surrogate for ultrasonography, because levels correlate closely with antral follicle count (AFC) in several investigations (3–6). AMH has been reported to be two- or three-fold higher in serum from women with PCOS than in women with normal ovaries (7). There is evidence that higher serum AMH levels reflect a more severe phenotype of PCOS (8–11). However, a negative association with lower circulating AMH and higher body mass index (overweight/obesity) in PCOS has also been observed and is likely to contribute to this correlation (9,11,12). This is in line with observations on the population in the present study (6). AFC and ovarian volume, like AMH, may indicate the severity of the syndrome. Classic PCOS, defined as PCO, hyperandrogenism, and oligo-/amenorrhea, presented higher AFCs and larger ovarian volumes compared with non-classic PCOS, defined as PCO and either hyperandrogenism or oligo-/amenorrhea, also after adjustment for age and body mass index (6).

The first-line treatment for women with PCOS is lifestyle interventions (diet, exercise, behavioral, or combined treatments), which has been demonstrated to reduce ovulatory dysfunction, improve body composition, reduce hyperandrogenism, and reduce insulin resistance in women with PCOS (13–15). Interestingly, AMH decreased after exercise in women with PCOS but not in controls in a small pilot trial (16). There are few studies on lifestyle interventions and ovarian morphology in humans. In a study on eight overweight or obese oligo/amenorrheic women with PCOS and 13 controls, using magnetic resonance imaging (MRI), there was a 15% decrease in AFC after 16 weeks of aerobic exercise training independent of changes in body composition, but no change in ovarian volume (17). Hence, whether exercise treatment affects AMH levels and ovarian morphology in women with PCOS remains to be elucidated.

Acupuncture is emerging as a nonpharmacological treatment, reducing hyperandrogenism and increasing menstrual and ovulation frequency, and superior to exercise, in women with PCOS (18–21). Two randomized controlled trials (RCT) evaluated the effect of acupuncture for ovulation induction and changes in endocrine measures including circulating AMH (20,22). One of these studies found no changes in circulating AMH caused by acupuncture with low-frequency electrical stimulation, so called electro-acupuncture (EA) treatments (22). They did show that baseline AMH was strongly correlated with ovulation frequency during the 5-month clinical trial protocol, lending further evidence that AMH could become a potential marker for the severity of the syndrome (22). The other RCT demonstrated that low-frequency EA treatments in lean/overweight women with PCOS resulted in a higher ovulation frequency and lower circulating sex steroids and inhibin B compared with an attention control group, but no significant change in circulating AMH levels although tendencies (20). Interestingly, one previous study reported a significant decrease in ovarian volume measured by transvaginal ultrasonography after acupuncture, whereas AFC was not reported (21). These findings are supported by an experimental study on different rat PCOS-models; acupuncture and exercise improved ovarian morphology, as reflected in a higher proportion of healthy (non-atretic) antral follicles (23).

Taken together, there are few studies evaluating the effect of acupuncture and exercise on circulating AMH and ovarian morphology including AFC and ovarian volume. We have previously shown that EA and exercise reduce circulating sex steroids and increase menstrual frequency when compared with no intervention in women with PCOS (18). Here, we therefore test the hypothesis, in secondary analyses, that acupuncture and exercise decrease AMH, AFC, and ovarian volume to a greater extent than no treatment.

Material and methods

Women with PCOS were recruited between November 2005 and January 2008 by advertising in the local community, as described by Jedel et al. (18). PCOS was defined according to modified Rotterdam criteria, i.e. ultrasound-verified PCO morphology (12 or more follicles 2–9 mm and/or ovarian volume >10 mL in one or both ovaries) with oligo-/amenorrhea and/or clinical signs of hyperandrogenism (hirsutism or acne) (1). Oligomenorrhea was defined as an intermenstrual interval >35 days and fewer than eight menstrual bleedings in the past year. Amenorrhea was defined as absent menstrual

Key Message

Repeated low-frequency electro-acupuncture treatment may reduce serum anti-Müllerian hormone levels and ovarian volume in women with polycystic ovary syndrome.

bleeding in the past 90 days. Hirsutism was defined as a Ferriman–Gallwey score ≥8. Acne was determined by an affirmative answer to the question "Do you have excessive acne?". Exclusion criteria were age <18 or >38 years, any pharmacological treatment within 12 weeks, pregnancy, breastfeeding within 24 weeks, and known cardiovascular disease or endocrine disorders other than PCOS (18). All participants gave oral and written consent. This is a secondary analysis of a prospective, randomized study that was conducted at the Sahlgrenska Academy/University Hospital in Göteborg, Sweden, in accordance with the Declaration of Helsinki and was approved by the Ethical Review Board in the Västra Götaland region (18). The study was registered at ClinicalTrials.gov (identifier NCT00484705).

Participants were randomized to acupuncture with combined manual and low-frequency electrical stimulation (EA), physical exercise, or no intervention in a 2:2:1 ratio. Computer-generated randomization within each stratum was conducted by using permuted blocks of five, and was concealed until interventions were assigned. To ensure equal proportions of age and body mass index in each study arm, randomization was stratified by these variables. Investigators analyzing data were not involved in the randomization procedure and were blinded to treatment allocation when analyzing data. After randomization, each participant underwent a 12-week period of observation of menstrual bleeding pattern, followed by a baseline assessment, 16 weeks of intervention, and 16 weeks of follow up. Details of the intervention have previously been described (18,24).

Acupuncture

Women in the low-frequency EA group received 30 min of treatment twice weekly for 2 weeks, once weekly for 6 weeks, and once every other week for 8 weeks (in total, 14 treatments) by a physical therapist educated in Western medical acupuncture. Disposable stainless-steel needles (Hegu Xeno, Hegu Svenska, Landsbro, Sweden; length 30/50 mm, diameter 0.32 mm) were inserted bilaterally in acupuncture points in abdominal muscles and in muscles below the knee located in somatic segments corresponding to ovarian innervations, see Jedel et al. for details (18). These needles were electrically stimulated at a frequency of 2-Hz burst frequency (CEFAR ACUS 4; Cefar-Compex Scandinavia, Malmö, Sweden); the intensity of stimulation was adjusted to produce local muscle contractions without pain or discomfort. Needles were also placed bilaterally in acupuncture points in muscles in the hand and in lower arm and were stimulated manually by rotation until needle sensation reflecting activation of sensory afferents, every 10 min.

Physical exercise

The physical exercise program consisted of aerobic exercise for 16 weeks at a self-selected pace, described as "faster than normal walking at a pace that could be sustained for at least 30 min and at least 3 days weekly" (18). Exercise was self-monitored and consisted of brisk walking, cycling, jogging, or any equivalent aerobic exercise. Participants received weekly telephone calls to provide support on increasing exercise. All exercise was in addition to daily physical activity.

No intervention

Women in the no intervention group, and also in the acupuncture and exercise group, received oral information about the benefits of regular physical exercise. Participants could call the study coordinator at any time.

Outcome measurements

Because most women with PCOS had oligo-/amenorrhea, the examination day was chosen independently of cycle day. Serum AMH was recorded at baseline, after 16 weeks of intervention (i.e. within 1 week after the last treatment), and at follow up after 32 weeks. AFC and ovarian volume were assessed by MRI at baseline and at follow up at 32 weeks.

Blood samples for analyses of AMH were drawn in the morning after an overnight fast independent of cycle day because the majority of women had irregular cycles. Serum AMH levels were measured by the enzyme immunoassay AMH-EIA (Beckman Coulter Immunotech, Bromma, Sweden) at an accredited laboratory at the Department of Clinical Chemistry, Sahlgrenska University Hospital. Serum AMH levels were measured by the enzyme immunoassay (Beckman Coulter Immunotech; limit of detection, 7.14 pmol/L, with an intra-assay coefficient of variation of 12.3% and inter-assay coefficient of variation of 14.2%).

MRI was performed with a 1.5-Tesla scanner (Intera; Philips Medical Systems, Amsterdam, the Netherlands). Technical details have previously been described (6,25). In brief, for ovarian morphological imaging, pelvic multislice T2-weighted turbo spin-echo acquisitions were performed in transaxial, sagittal, and coronal planes. The MRI data were transferred to an image workstation (Centricity Workstation Radiology RA 600; GE Healthcare, Wauwatosa, WI, USA). Visible (antral) ovarian follicles were manually counted in at least two planes. MRI-derived AFC was defined as all visible (cystic) follicles in the size interval 1–9 mm. Total ovarian volume was

semi-automatically measured in three dimensions at an advanced workstation (Advantage Windows Analysis Station, GE Healthcare). The volume of cysts >2 cm was calculated in the same way and subtracted from total ovarian volume. All MRI scans were interpreted by an experienced radiologist (HL), who was blinded to group, clinical information, and ultrasonographic findings. The three-dimensional data were obtained by a trained medical student, supervised by an experienced radiologist (HL).

Statistical analyses

Data are presented as median and range. Sample size calculation has previously been presented for the primary outcome measure (18). Intrapatient morphological features of the left and right ovaries did not differ and are presented as mean for each subject. Data were analyzed according to the intention-to-treat principle. To evaluate changes between baseline and weeks 16 and 32 respectively, missing data were replaced by carrying forward the last observation. Dropouts between randomization and baseline assessments were excluded from the intention-to-treat analyses. Most data were not normally distributed, therefore between-group differences were assessed with the non-parametric Kruskal–Wallis test followed by the Mann–Whitney *U*-test; the primary comparison was between EA and exercise. Within-group changes were analyzed with the Wilcoxon Signed Rank test. Associations between change in menstrual frequency and change in circulating AMH in each group were estimated by Spearman's rank correlation. A value of $p < 0.05$ was considered significant. Statistical analyses were conducted with PASW Statistics version 21.0 for Windows (IBM Corp, Armonk, NY, USA).

Results

The present study comprises secondary analyses from the original trial, and details of participant flow through the study and phenotypic distributions have been described elsewhere (18). In brief, after an initial telephone interview screening, 100 remaining women underwent a gynecological examination, including transvaginal ultrasonography (HDI 5000 ATL, Bothell), to confirm the obligate inclusion criterion of PCO morphology. Eighty-four women met the inclusion criteria and were randomized to three groups: EA ($n = 33$), exercise ($n = 34$), and no intervention ($n = 17$). During the initial 12-week observation period, there were 10 dropouts; the remaining 74 women (29 in the EA group, 30 in the exercise group, and 15 in the no intervention group) underwent baseline assessments, started treatment, and were included in the intention-to-treat analyses. Due to limited availability of MRI examination time, there was a time span between the serum sampling of AMH and MRI examination in which there were further dropouts, resulting in a smaller sample size of 38 with MRI data (17 in the EA group, 14 in the exercise group, and 7 in the no intervention group). The groups were comparable at baseline regarding all measurements (Table 1).

Between baseline and week 16, i.e. revealing a short-term effect, serum AMH decreased significantly in the EA group. The AMH decrease remained significant at follow up, suggesting a long-term effect (Table 2). In addition to AMH, there was a significant decrease in ovarian volume between baseline and follow up in the EA group, and of AFC in the exercise group (Table 3).

Serum AMH levels decreased by 17.5% in the EA group between baseline and week 16, and differed from a 7.5% AMH increase in the exercise group ($p = 0.002$) but

Table 1. Baseline characteristics.

Variable	Low-frequency electro-acupuncture ($n = 29$)	Physical exercise ($n = 30$)	No intervention ($n = 15$)	*p*-value between groups
Age (years)	28.0 (22 – 37)	29.5 (21 – 37)	30.0 (21 – 36)	0.893
Body mass index (kg/m^2)	24.8 (18.2 – 47.4)	26.6 (20.4 – 44.3)	24.5 (20.6 – 37.6)	0.888
Anti-Müllerian hormone (pmol/L)	66.0 (25.3 – 194.1)	67.0 (20.6 – 262.5)	82.8 (16.9 – 273.5)	0.776

MRI[a] variable	Low-frequency electro-acupuncture ($n = 17$)	Physical exercise ($n = 14$)	No intervention ($n = 7$)	*p*-value between groups
Ovarian volume (mL)	11.4 (7.7 – 21.0)	12.2 (6.1 – 21.6)	14.7 (8.4 – 23.9)	0.667
Antral follicle count[b]	44.5 (17 – 79)	39.8 (18 – 92)	59.0 (18 – 96)	0.493

Values are median (range). Between-group differences at baseline were determined by Kruskal–Wallis test.
MRI, magnetic resonance imaging.
[a]Derived ovarian volumes do not include cysts ≥2 cm.
[b]1–9 mm in size.

Table 2. Change in anti-Müllerian hormone (AMH) from baseline to week 16 and 32.

	Low-frequency electro-acupuncture (n = 29)			Physical exercise (n = 30)			No intervention (n = 15)			
Variable	Median (range)	Change (%)	p-value[a]	Median (range)	Change (%)	p-value[a]	Median (range)	Change (%)	p-value[a]	p-value between groups[b]
AMH (pmol/L); week 16	−3.8 (−108.4 – 8.4)	−17.5	<0.001	0 (−41.5 – 178.9)	7.5	0.903	−7.1 (−47.9 – 33.4)	−1.9	0.311	0.010
AMH (pmol/L); week 32	−2.6 (−103.4 – 15.1)	−15.0	0.004	0 (−47.9 – 114.9)	−0.2	0.412	−6.4 (−47.9 – 57.3)	−7.4	0.133	0.169

Values are change in median (range), and percentage.
[a]Within-group changes were determined by Wilcoxon Signed Rank test.
[b]Between-group differences at weeks 16 and 32, respectively, were determined by Kruskal–Wallis test. In case of significance (week 16), it was followed by Mann–Whitney *U*-test, which demonstrated a difference between low-frequency electro-acupuncture and physical exercise ($p < 0.002$).

Table 3. Changes in magnetic resonance imaging variables from baseline to week 32.

	Low-frequency electro-acupuncture (n = 17)			Physical Exercise (n = 14)			No intervention (n = 7)			
Variable	Median (range)	Change (%)	p-value[a]	Median (range)	Change (%)	p-value[a]	Median (range)	Change (%)	p-value[a]	p-value between groups[b]
Ovarian volume[c] (mL)	−1.3 (−1.7 – 4.5)	−8.5	0.015	−0.3 (−3.0 – 4.2)	−1.1	0.551	–1.1 (−4.2 – 4.5)	−0.1	0.237	0.570
Antral follicle count[d]	−2.0 (−11.0 – 22.0)	−3.8	0.266	−6.3 (−3.5 – 31.5)	−11.7	0.010	−0.5 (−13.0 – 45.5)	−6.3	0.735	0.347

Values are change in median (range), and percentage.
[a]Within-group changes were determined by Wilcoxon Signed Rank test.
[b]Between-group differences at follow up were determined by Kruskal–Wallis test.
[c]Magnetic resonance imaging-derived ovarian volumes do not include cysts ≥2 cm.
[d]1–9 mm in size.

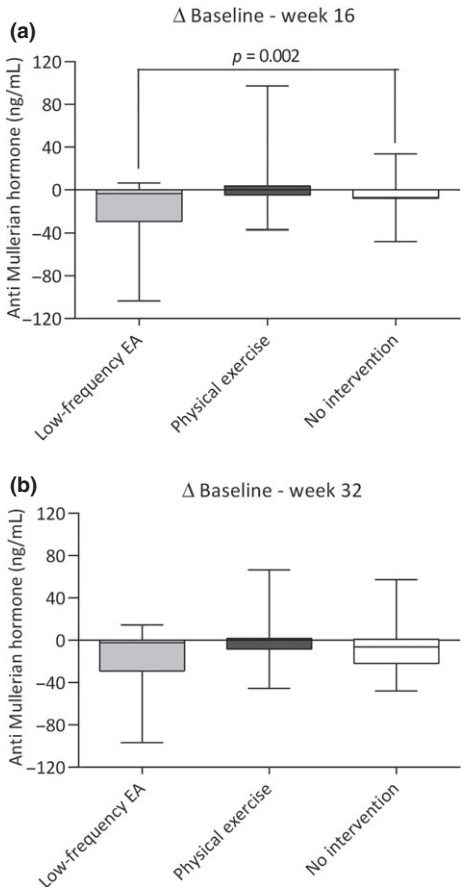

Figure 1. (a) Between-group differences in serum anti-Müllerian hormone levels, determined at baseline and at 16 weeks, after 16 weeks of intervention. The *p*-value was determined by Mann–Whitney *U*-test. (b) Between-group differences in serum anti-Müllerian hormone levels, determined at baseline and at 32 weeks, after 16 weeks of intervention. EA, electro-acupuncture.

not from an 1.9% AMH decrease in the no intervention group (Figure 1a). At follow-up week 32, the decrease from baseline of AMH did not remain significantly different compared with the levels in the other groups (Figure 1b) (Table 2). From baseline to follow up, the change of MRI-derived morphological variables was not different between the three groups (Table 3).

Changes in bleeding pattern (baseline – after 16 weeks of treatment) was positively associated with a decrease in AMH (R_s −0.558, $p = 0.009$). There was no correlation between baseline and follow up in the acupuncture group or in the exercise and no treatment group at any time point. Exercise frequency and physical fitness have been described in detail previously (18). No difference between the groups in self-reports of physical exercise frequency or maximal aerobic capacity ($V\mathrm{O}_{2\mathrm{max}}$) was reported. No adverse events were noted.

Discussion

This is the first study to demonstrate that low-frequency EA reduces serum AMH levels after 16 weeks of treatment, as shown in a secondary analysis of our RCT. The reduction of circulating AMH remained lowered at follow-up 4 months after the last treatment, but did not differ from the exercise group. In addition to AMH, the ovarian volume decreased within the EA group and the AFC decreased within the physical exercise group between baseline and follow up.

There is increasing evidence that serum AMH levels and AFC reflect ovarian reserve and the severity of PCOS (8–11,22,26–29). The risk of anovulation is increased with increased number of antral follicles in the ovaries. Several studies have reported a positive correlation between AFC and serum concentrations of androgens (4,6,7,12,30,31). These observations support the hypothesis that high intra-ovarian androgen levels play a major role in the disturbed folliculogenesis of PCOS, causing follicular arrest and increased ovarian AMH production (32). Serum AMH levels correlate positively with androgen levels, and higher levels of AMH have been observed in hyperandrogenic women with PCOS compared with those who are normoandrogenic (6–9,11,16,33–35).

There is evidence that the local follicle-to-follicle signaling of AMH and other regulators is abnormal and contributes to the disordered folliculogenesis (36). We have previously reported that repeated low-frequency EA reduce circulating androgen levels in women with PCOS compared with both exercise and an untreated control group (18). This may in part explain the significant reduction of AMH levels observed in the EA group. Since serum AMH levels and AFC are strongly interrelated, it is somewhat surprising that we did not observe a significant reduction in AFC in the EA group. Although not statistically significant, there was a trend of decreasing AFC over time within the EA group. It can be speculated that with a larger sample size it might have been significant. AMH did not change by exercise intervention in the present study, but within the exercise group there was a reduction of AFC between baseline and follow up. This is in accordance with Redman et al., who reported decreased AFC in overweight women with PCOS after 16 weeks of exercise (17). An increase in insulin sensitivity was also observed, and the authors speculate that the reduction in AFC may be explained by this finding (17). However, no change in insulin sensitivity was observed in the intervention groups in our study population, which may be explained by a lower exercise intensity compared with the Redman study (24). Interestingly, the ovarian volume was significantly decreased within the EA group. The ovarian volume reduction observed after EA may be explained by the reduction in circulating androgens

and equivalent reduction of androgen-producing ovarian stroma (6,12). Of note is that the decrease in AMH from baseline to after 16 weeks of intervention was associated with increases in menstrual frequency (18).

Women with PCOS need some kind of long-standing treatment. Because the efficacy and patient compliance with existing treatments, such as oral contraceptives and metformin, are unsatisfactory and associated with negative side effects, there is a clear need for novel treatment options that can complement or replace current treatments. That physical exercise and acupuncture may modulate disturbed reproductive, metabolic, neuroendocrine, and coagulation factors in women with PCOS is in accordance with previous clinical experience and reports (18,20,21,24,37–42).

Previous studies on lifestyle modifications including exercise and diet and their impact on AMH in women with PCOS have reported contradictory results (16,20,22,34,43). In a pilot trial, exercise during 12 weeks decreased AMH in anovulatory women with PCOS (16). Interestingly, Nybacka et al. reported decreased serum AMH levels by diet intervention in association with decreased androgen levels and improvement of menstrual cyclicity in overweight/obese women with PCOS, whereas physical exercise did not affect AMH (43). All women fulfilled all three Rotterdam criteria of PCOS and had a relatively high mean baseline AMH level of 70 pmol/L. In another study, including 52 overweight and obese women with PCOS, a 20-week weight loss program resulted in improved reproductive function but no change in AMH levels (34). In that study, PCOS was diagnosed according to the Rotterdam definition (i.e. two of the three criteria are sufficient for diagnosis) and a mean baseline AMH level of 28 pmol/L was reported, which may be a possible reason for the unchanged AMH levels after weight loss. In the present study, the mean baseline level of AMH for all participants was 89 pmol/L, so comparable to the high pre-treatment level in the RCT by Nybacka et al., and our results showing AMH reduction after intervention with EA but not after exercise correspond to their results.

A strength of the present study was the assessment of ovarian morphology by MRI. In a previous study, we found this imaging modality more precise in counting small antral follicles compared with transvaginal ultrasonography (25). In addition, MRI has the ability to determine ovarian volume also in irregularly or non-spherically shaped ovaries (25). Other strengths were the RCT design, patient selection (recruitment from the community), absence of co-interventions, and the relatively long follow up (4 months) after the last treatment.

A potential limitation is that our results are based on a secondary analysis, as the study was not designed or powered to address these particular issues. Because multiple comparisons have already been made with this same study set in the previous publications (18,24), there is a risk of false-positive findings (i.e. type I errors). For this reason, and because of the limited number of participants, the results need to be substantiated by prospective larger studies with the current issues as primary objectives. Another limitation, as in all acupuncture and exercise studies, is that it is difficult to control for a placebo effect, as we have discussed in previous reports (18,20,24). A further limitation is that MRI was not also performed after 16 weeks of intervention. Thereby, only more long-term or long-lasting effects of the intervention could be demonstrated, while important short-term mechanisms or effects might have been missed. Further, since the follow-up time was 32 weeks, we cannot exclude that the decline in AMH may to some extent be explained by the natural regression of AMH over time.

In conclusion, repeated low-frequency EA treatment reduces serum AMH levels immediately after 16 weeks of intervention (a short-term effect), and reduces ovarian volume at follow-up 16 weeks after end of intervention (a long-term effect) as determined by MRI. Physical exercise did not influence AMH levels or ovarian volume. Despite a significant within-group decrease in AFC at follow up after physical exercise, effects of exercise compared with no intervention could not be proven.

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Research Article

Effect of Acupuncture on Premature Ovarian Failure: A Pilot Study

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To investigate the effect of acupuncture on women with premature ovarian failure (POF), prospective consecutive case series study was applied. 31 women with POF were included; all patients were treated with acupuncture once every other day, three times a week for three months. Acupoints, GV 20, GV 24, GB 13, CV 3, CV 4, BL 23, BL 32, ST 25, ST 28, ST 29, ST 36, SP 6, KI 3, and LR 3, were selected. Serums FSH, E_2, and LH level, Self-Rating Anxiety Scale (SAS), and Kupperman score were measured at baseline and at the end of treatment; the menstrual cycle was recorded during one-month follow-up. Compared with baseline, patients' serums FSH and LH were decreased, E_2 was increased, and SAS score and Kupperman score were decreased. Four patients resumed menstrual cycle after treatment and two resumed during follow-up. No serious adverse events were found in all patients. The results indicate that acupuncture may decrease serums FSH and LH level, raise serum E_2 level, relieve anxiety, reduce mental stress, and improve the menopausal symptoms.

1. Introduction

Premature ovarian failure (POF) is a common cause of infertility in women and is characterized by amenorrhea before the age of 40. Menopause before the age of 40 is considered to be premature and is nonphysiological disorder. POF, also known as premature menopause or premature ovarian insufficiency, is defined by the presence of menopausal-level serum follicle-stimulating hormone (FSH) in women younger than 40 years [1–3]; the mean (±SD) age of natural menopause is 50 ± 4 years [4]. This syndrome is associated with the symptoms and metabolic effects of sex steroid deficiency, as well as the emotional sequelae experienced by couples who have difficulty in conceiving a pregnancy. POF is a disorder affecting approximately 1% of women <40 years, 1/1,000 women by the age of 30, and 1/10,000 women by the age of 20.

Women with POF have been reported to have diminished general and sexual well-being, are less satisfied with their sexual lives, have increased risk for low bone density, earlier onset osteoporosis and fractures, impaired endothelial function, earlier onset of coronary heart disease, and increased cardiovascular mortality and total mortality, and have more anxiety, depression, somatization, sensitivity, hostility, and psychological distress than normal women [5–7].

POF was not nominally recorded in Traditional Chinese Medicine (TCM). However, according to TCM theory, POF can be pertained to amenorrhea according to the clinical manifestations. As early as in 1237 A.D., the first book about gynaecology and obstetrics of Chinese Medicine, *The Complete Book of Effective Prescriptions for Diseases of Women*, said that acupuncture and Chinese Herbal Medicine usually led to satisfying symptom relieving effects in treating some gynaecology disorders, such as endometriosis, infertility, amenorrhea, and menopausal syndrome [8]. In recent studies, acupuncture has been found effective in reducing hot flashes of bilaterally ovariectomized patients [9] and women undergoing perimenopause and menopause [10–12] and significantly improving serum estradiol (E_2) level and

Table 1: Acupoints used in this study.

Prescription	Acupoints
Prescription 1	CV 3 (Zhongji), CV 4 (Guanyuan), ST 25 (Tianshu), ST 28 (Shuidao), ST 29 (Guilai), and LR 3 (Taichong) GV 20 (Baihui), GV 24 (Shenting), GB 13 (Benshen), ST 36 (Zusanli), SP 6 (Sanyinjiao), and KI 3 (Taixi)
Prescription 2	BL 23 (Shenshu), BL 32 (Ciliao) GV 20 (Baihui), GV 24 (Shenting), GB 13 (Benshen), ST 36 (Zusanli), SP 6 (Sanyinjiao), and KI 3 (Taixi)

Kupperman scores of perimenopause patients [13]. Acupuncture also has been proved to be effective in improving pregnancy outcomes in women undergoing *in vitro* fertilization (IVF) from summarized data of several previously published meta-analysis and lots of clinical trials [14, 15]. Although the therapeutic mechanisms of acupuncture in the above-mentioned disorders are not yet to be fully understood, a plausible hypothesis is that acupuncture may influence related hormone levels.

However, there is less evidence on the effectiveness of acupuncture for patients with POF. This study, therefore, was designed to assess the feasibility and safety of acupuncture for POF patients on the basis of TCM theory, which includes local and distal acupoints to reinforce liver and kidney, to regulate Qi and Blood, and to adjust mental activity.

2. Materials and Methods

2.1. Study Design. This was a prospective consecutive case series study performed at the Hospital of Acupuncture and Moxibustion of China Academy of Chinese Medical Sciences. Participants received 3 sessions of acupuncture treatment, delivered 3 times a week for 3 months. The data from patients include the serums FSH, E_2, and luteinizing hormone (LH) level, Self-Rating Anxiety Scale (SAS) [16], and Kupperman score [17], (The SAS and Kupperman score have been validated and widely used in Chinese population [18, 19].) The menstrual cycles were recorded during one-month follow-up. Adverse events were tracked for 3 months from initial acceptance of acupuncture treatment to the end of treatment.

The institute ethics committee approved this treatment protocol for women with POF (Institute of Acupuncture and Moxibustion Beijing (ethics) approval number: 20111117), and all patients signed informed consent before study participation. Acupuncture was performed by one therapist with more than 20 years of experience and Chinese medicine practitioner license from the Ministry of Health of the People's Republic of China. Data coordination, monitoring, analysis, and source verification were done by the researchers who did not take part in the clinical treatment. Figure 1 shows the study design.

2.2. Study Participants. Present study recruited patients through the following ways: (1) publish recruiting advertisement on the website of the Hospital of Acupuncture and Moxibustion of China Academy of Chinese Medical Sciences; (2) publish recruiting notification on the largest Chinese doctor information website; (3) place recruiting advertisement in registration hall of our hospital. Informed consent was obtained before participant enrollment according to a clinical trial protocol. Recruitment was performed from December 2011 to January 2013, and 31 cases of POF were recruited.

Inclusion criteria were as follows: (1) the patients meet the POF diagnosis criteria, mainly with amenorrhea for four months or longer and FSH above 40 IU/L as detected on at least two occasions with at least one month apart [20–22]; (2) the patients' age is from 18 to 40; (3) before treatment, all patients had gone through one-month baseline evaluation period during which they stopped all medications influencing reproductive hormones; (4) patients were advised and agreed not to use these medications during study. Exclusion criteria were as follows: (1) bilateral oophorectomy, gonadal dysgenesis, and chromosomal abnormalities; (2) ovarian failure caused by radiotherapy and/or chemotherapy of cancer; (3) autoimmune diseases and/or receiving hormones or immunosuppressant drugs; (4) serious primary diseases of cardiovascular, liver, kidney, and hematopoietic system; (5) having no desire to participate in the research.

2.3. Interventions

Acupuncture Needles. Sterile, silver-handle, prepacked needles (HanYi single-use acupuncture needle, made in Tianjin HuaHong Medical Co., Ltd.) without guide tubes (size 0.25 mm × 25 mm, 0.25 mm × 40 mm, and 0.30 mm × 75 mm).

All acupoints were selected and localized according to WHO Standardized Acupuncture Point Location [23] (Table 1). Needles of the size of 0.25 mm × 25 mm were inserted horizontally into GV 20 (Baihui), GV 24 (Shenting), and the bilateral GB 13 (Benshen) with a depth of 20 mm and inserted perpendicularly into the bilateral KI 3 (Taixi) and LR 3 (Taichong) with a depth of 20 mm. 0.25 mm × 40 mm size needles were inserted perpendicularly into CV 3 (Zhongji), CV 4 (Guanyuan), the bilateral ST 25 (Tianshu), ST 28 (Shuidao), ST 29 (Guilai), ST 36 (Zusanli), and SP 6 (Sanyinjiao) with a depth of 30–35 mm. 0.30 mm × 75 mm size needles were inserted obliquely into the bilateral BL 32 (Ciliao, second sacral foramina) with a depth of 50–60 mm.

For acupuncture at BL 32 (Ciliao, second sacral foramina), patients should have a strong soreness sensation which radiates to the lower abdomen. For the other points, mild reinforcing-reducing method was used, and all patients should have the "De Qi" sensation (in which patients experience a radiating feeling considered to be indicative of effective needling).

The needles were administered for a maximum of 20–30 minutes, and acupuncture treatment was administered once every other day (two prescriptions were used alternatively,

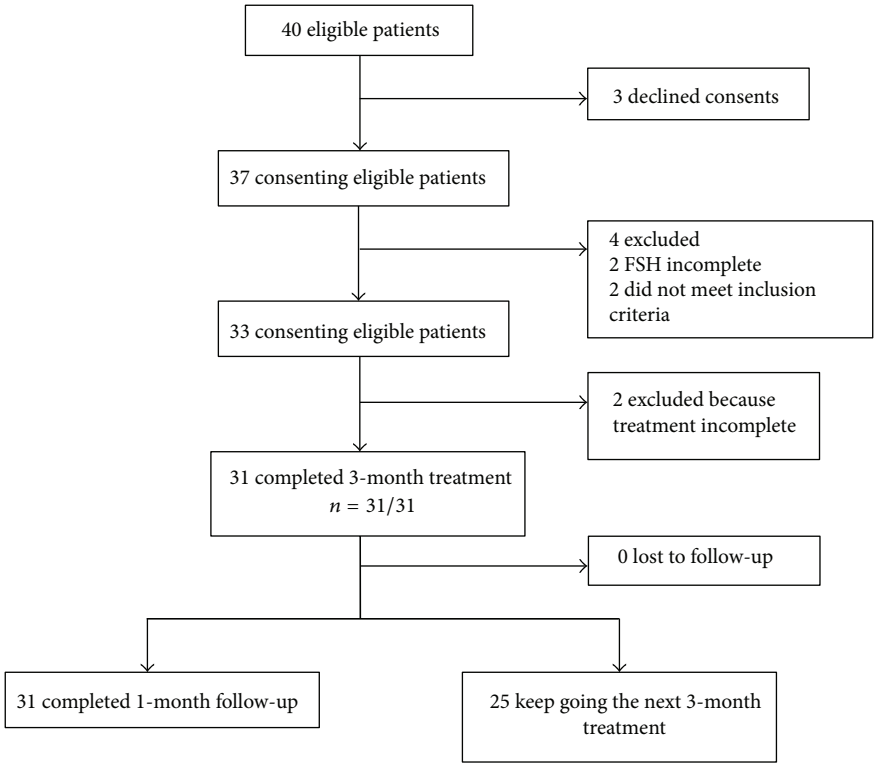

Figure 1: Flow chart of study.

prescription 1 twice a week and prescription 2 once a week) for three months.

2.4. Study Outcomes. The data collected including the serums FSH, E_2, and LH level, SAS, and Kupperman score, at baseline and after 3-month treatment. Additionally, patients' menstrual cycles were recorded according to individuals' reports during one-month follow-up. Adverse events were tracked for 3 months from initial acceptance of acupuncture treatment to the end of treatment.

2.5. Statistical Analysis. All analyses were done with SPSS software package (Version 17.0) using before and after measurements. Baseline characteristics of the patients were analyzed with conventional group descriptive statistics. t-test was used firstly for serums FSH, E_2, and LH level, SAS, and Kupperman score. For the measurement data fitting normal distribution, using Two-Related-Samples Tests. If the measurement data did not fit the normal distribution, using Two-Related-Samples Tests Wilcoxon.

3. Results

3.1. Participant Flow. From December 2011 to May 2013, a total of 40 patients with POF visited the Hospital of Acupuncture and Moxibustion of China Academy of Chinese Medical Sciences seeking for acupuncture treatment. Of these patients, nine were excluded from the study with the following reasons: three patients declined to participate; two had incomplete FSH data; two did not meet inclusion criteria; two did not complete the whole session of treatment. (One patient moved to another city for job reason. The other one could not guarantee three times treatment per week after three-week treatment, because of her difficulties in asking for leave (Figure 1 and Table 2).)

Table 2: Demographic and clinical characteristics at baseline.

Variable	N (%) or mean ± SD
Mean age, age	35 ± 4
Mean weight, kilogram	53.7 ± 7
Mean age at menarche, age	14 ± 1
History of amenorrhea, month	8 ± 6
History of pregnancy, n	15 (48)
History of abortion, n	13 (42)
History of delivery, n	5 (16)
Fertility request, n	26 (84)

Of these 31 patients, 25 had not been treated by acupuncture in the past, and six had been treated by acupuncture at least one month ago. And in the six, there were four received acupuncture treatment for cervical spondylosis, and two received acupuncture treatment for menstrual problem.

3.2. Baseline Characteristics. The baseline characteristics of the participants are shown in Table 2. The mean age at baseline was 35 years (SD = 4; range, 24~40). The mean history of amenorrhea was 8 months (SD = 6; range, 4~25). 26 of the 31 participants were with fertility request. No

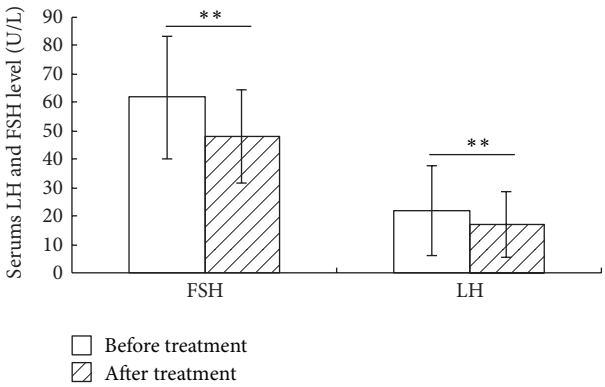

Figure 2: The serums LH and FSH level before and after acupuncture treatment. Both the serums FSH and LH level were reduced significantly after acupuncture treatment for 3 months ($P = 0.001$, $P = 0.001$, resp., $n = 31$) ($^{**}P < 0.01$).

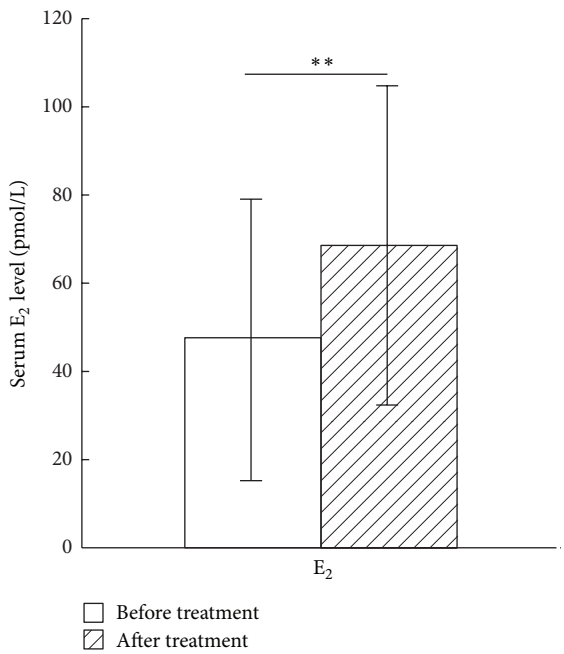

Figure 3: The serum E_2 level before and after acupuncture treatment. The serum E_2 level was reduced significantly after acupuncture treatment for 3 months ($P = 0.001$, $n = 31$) ($^{**}P < 0.01$).

participant reported the use of hormone therapy or Chinese herbal therapy at baseline or during the entire observation period.

3.3. Effects of Acupuncture on Serums FSH and LH Level. Changes in mean serum FSH level from baseline to 3 months after final acupuncture treatment session are presented in Figure 2. Two-Related-Samples Tests Wilcoxon showed a significant reduction in the average serum FSH level at the end of treatment ($Z = 4.68$, $P = 0.001$).

Changes in mean serum LH level from baseline to 3 months after the last time acupuncture treatment session are presented in Figure 2. Two-Related-Samples Tests showed

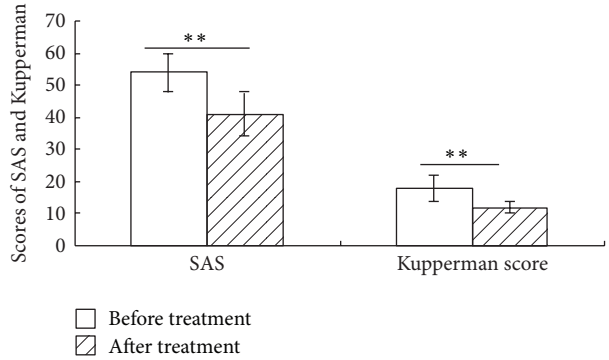

Figure 4: The scores of SAS and Kupperman before and after acupuncture treatment. Both the SAS and Kupperman scores were reduced significantly after acupuncture treatment for 3 months ($P = 0.000$, $P = 0.000$, resp., $n = 31$) ($^{**}P < 0.01$).

a significant decrease in the average serum LH level at the end of treatment ($t = 5.519$, $P = 0.001$).

3.4. Effects of Acupuncture on Serum E_2 Level. Changes in mean serum E_2 level from baseline to 3 months after final acupuncture session are presented in Figure 3. Two-Related-Samples Tests Wilcoxon showed a significant reduction in the average serum E_2 level at the end of treatment ($Z = 4.48$, $P = 0.001$).

3.5. Effects of Acupuncture on the Anxiety State Assessed by SAS and the Perimenopausal Syndrome via Kupperman Score. Figure 4 showed that, during one-month baseline evaluation period, patients' SAS score was 54 ± 6. Two-Related-Samples Tests Wilcoxon showed a significant reduction to 41 ± 7 after 3-month acupuncture treatment ($Z = 4.82$, $P = 0.000$).

Figure 4 showed that, during one-month baseline evaluation period, patients' Kupperman score was 18 ± 4. Two-Related-Samples Tests Wilcoxon showed a significant reduction to 12 ± 2 after 3-month acupuncture treatment ($Z = 4.71$, $P = 0.000$).

3.6. Effects of Acupuncture on Menstrual Cycle. After 3-month treatment, six patients had improvement on menstruation (6/31, 19.4%). Four of them had menstruation after treatment, and two of them experienced menstruation during the one-month follow-up. Of the 6 patients who regained menstruation, 2 patients regained normal color, duration, and volume of period, and 4 patients had decreased menstrual flow but with normal color and duration as compared with normal menstrual bleeding.

3.7. Safety. During the 3-month treatment, two adverse events were reported by 2 patients (1 small haematoma and 1 needling pain after treatment). No serious adverse events were documented.

4. Discussion

Although POF was not nominally recorded in TCM, its clinical manifestations can be classified into amenorrhea

according to TCM theory. The TCM etiology of amenorrhea includes insufficient transformation of blood of the spleen and stomach, severe consumption of yin blood and exhaustion of blood source, and blockage of blood stasis in the meridians and vessels due to retention of pathogenic factors in the uterus. In this study, local and distal acupoints with functions of reinforcing liver and kidney, regulating Qi and blood, and adjusting mental activity were selected according to the amenorrhea etiology of TCM theory.

The present results indicate that the acupuncture treatment can adjust patients' serums FSH, E_2, and LH level and improve patients' SAS score and Kupperman score. The serum FSH level dropped to 48 ± 16.6 IU/L from baseline to the end of treatment ($P = 0.001$), the serum E_2 level rose to 68.24 ± 36.15 pmol/L ($P = 0.001$), and the serum LH level dropped to 17.01 ± 11.66 ($P = 0.001$). The changes of hormones produced by acupuncture in present study were similar to the previous investigation using EA [24]. Modulation of serums FSH, E_2, and LH level may partially explain the effects of acupuncture in treating POF, which is also observed in acupuncture improving other gynecological disorders [25].

Four patients regained menstrual cycle after treatment and two during one-month follow-up period of this study. Such result of present study is similar to the gaining of menstruation in a top athlete reported in a Japanese acupuncture study [26].

Patients' psychological comorbidities, signs, and symptoms were objectively measured with standard questionnaires by SAS and Kupperman score. The SAS score decreased to 41 ± 7 ($P = 0.000$ as compared with baseline), and Kupperman score decreased to 12 ± 2 ($P = 0.000$ as compared with baseline). The change of SAS and Kupperman score indicated that acupuncture may relieve anxiety, reduce mental stress, and improve menopausal symptoms (including hot flashes, night sweats, vaginal dryness, and mood swings).

Although POF is most frequently idiopathic or caused by autoimmune disorders, genetic causes, infections or inflammatory conditions, enzyme deficiencies, or metabolic syndromes [27], it also related to high mental and psychological distress [7]. Such cooccurrence of physical and psychological illnesses is not only associated with poor treatment response, lower quality of life, and increased healthcare costs but also related to the mutual influence of physiological processes and psychological distress that contribute to the development of POF. Present study just revealed that the SAS and Kupperman score of patients were lowered after three months' treatment. This might be explained by the mind adjustment and mental improving effects of acupuncture [28, 29].

Nonetheless, this present study only included 31 patients; thus the result of the study may not well characterize the general response of women with POF receiving acupuncture treatments. With an open label prospective study design and no control group, researcher could not eliminate these confounding factors. Of the 31 patients, there were two patients had acupuncture treatment in other hospitals 1.5 and 2 months before participating in our study. Because the detailed information of their previous acupuncture treatment (like acupoints selected and stimulating methods applied) was not fully recorded, we could not compare those two acupuncture regimens. This is a methodological drawback of present study. Thus, the detailed relevant information about previous treatment of participants should be fully and meticulously recorded in future study. Meanwhile, in order to increase the objectivity and fairness of observational study, inclusion and exclusion criteria of future study should be stricter. Excluding those patients who have had other treatments may reduce confounding factors.

Commonly used acupuncture formulas for amenorrhea in China normally include acupoints with functions of reinforcing liver and kidney and regulating Qi and blood. Compared to the commonly used formulas, our acupuncture regimen added acupoints for adjusting mental activity. This may partially explain the different therapeutic effects between the two patients' previous formula and ours.

Although the significant changes of serums FSH, E_2, and LH level in this study were most likely due to acupuncture effects, those serum hormones' levels did still not reach the normal level after treatment. To test the therapeutic effectiveness of acupuncture, further randomized control trials are needed.

5. Conclusion

The present results showed the feasibility and safety of acupuncture for the treatment of POF in China. These findings suggest that acupuncture may decrease serums FSH and LH level, raise serum E_2 level, relieve anxiety, reduce mental stress, and improve the menopausal symptoms. No serious side effects were found.

Conflict of Interests

The authors declare that no competing financial interests exit.

Authors' Contribution

Yigong Fang conducted acupuncture treatment for all patients and had overall responsibility for the trial. Jinsheng Yang contributed to the study design. Yingru Chen collected the data and wrote the report, which was reviewed by all authors. Fei Wang collected data. Yingying Wang and Li Yang did the literature search for this study.

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