ACUPCOS: Acupuncture & PCOS – A Multi-Centres Randomised Controlled Trial

Chi Eung Danforn Lim[*,1,2], Xiping Luo[3], Xinlin Zhan[3] and Wu Shun Felix Wong[4]

[1]*South Western Sydney Clinical School, Faculty of Medicine, University of New South Wales, Australia*

[2]*Specialist Medical Services, Sydney Australia*

[3]*Guangdong Women's and Children's Hospital, Guangzhou, PR China*

[4]*School of Women's and Children's Health, Faculty of Medicine, University of New South Wales, Australia*

Abstract: *Background*: Polycystic ovarian syndrome (PCOS) is a prevalent and burdensome endocrine disorder. Acupuncture was proposed as a novel treatment. A range of preliminary results in favour of its use were found in published reports. This paper presents the first randomised controlled study on body acupuncture with Chinese medicine syndrome differentiation for PCOS. *Method*: A propective single blinded randomised controlled trial was being carried out from Jan 2008 to May 2009 at the Liverpool Hospital in Sydney Australia and Guangdong Women's & Children's Hospital in Guangzhou China. A total of 146 participants diagnosed with PCOS and Kidney Yang deficiency according Chinese medicine (CM) syndrome differentiation diagnoses were recruited. All participants were randomly assigned into a real acupuncture intervention group or sham acupuncture control group in a 2:1 ratio. *Results*: 141 subjects completed the study. A significant reduction in inter-menstruation interval was noted in intervention group, as well as in the level of FSH, LH, LH: FSH ratio, Progesterone and FAI were observed. *Conclusion*: It seems acupuncture has a role in restoring regular menstrual pattern as well as the hormonal profiles among women with PCOS. This effect is thought to be related to β-endorphin's modulatory effect on the hypothalamus-pituitary-adrenal (HPA) axis and direct influence on the serum level of FSH, LH, oestrogen and progesterone. Further research is needed in this area.

Keywords: Acupuncture, PCOS, Menstruation, Hormones.

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is a common endocrine disorder that affects 10-15% women [1]. Its manifestations include hirsutism, acne, infertility and increased risks of metabolic disorders, cardiovascular disease and malignancies [1]. These symptoms often pose life-long impact on well-being of affected women [1]. Conventional treatments of PCOS involve weight loss, lifestyle modification and hormonal treatment for hyperandrogenism and insulin resistance [2,3].

Acupuncture has been seen as a promising non-pharmacological candidate for the management of PCOS. It was postulated that acupuncture may ameliorate PCOS by modulating neuroendocrine patterns associated with insulin resistance [4]. Published reports of animal models and observational studies are found in favour of this hypothesis although it has never been tested in randomised controlled trial (RCT) [4,5]. This study was carried out in Jan 2008 - May 2009. As it was the first acupuncture RCT on PCOS with involvement of Chinese medicine syndrome differentiation, ovulation rate was not the primary outcome of this study but rather to elicit its physical response on menstruation return from amenorrhoea (menstrural rate) as well as its effect on hormonal changes for women with PCOS before and after acupuncture intervention.

METHOD

Study Sample

To achieve the power of 0.80, a sample size of 33 in each arms was needed. A total of 150 subjects were eventually able to recruit for this study. All subjects fulfilled the diagnostic criteria of Rotterdam Consensus [6] and all have Kidney yang deficiency syndrome as per Chinese medicine diagnosis. There were 2 drop outs from each arm and with this only 146 subjects completed the study. Details can be found at Figure **1** which outline the flow of the study. From these 146 subjects, 98 subjects were placed for the intervention group and 48 for the control group as to achieve a 2:1 ratio. Intervention group received placebo (sham) acupuncture treatment while the control groups received real body acupuncture treatment.

Study Design and Randomisation

This study occurred over 7 months which consisted of 1 month run in period, 3 months intervention period

*Address correspondence to this author at the P.O. Box 3256, Blakehurst, NSW 2221, Australia; Tel: +61 412 280 925; E-mail: celim@unswalumni.com
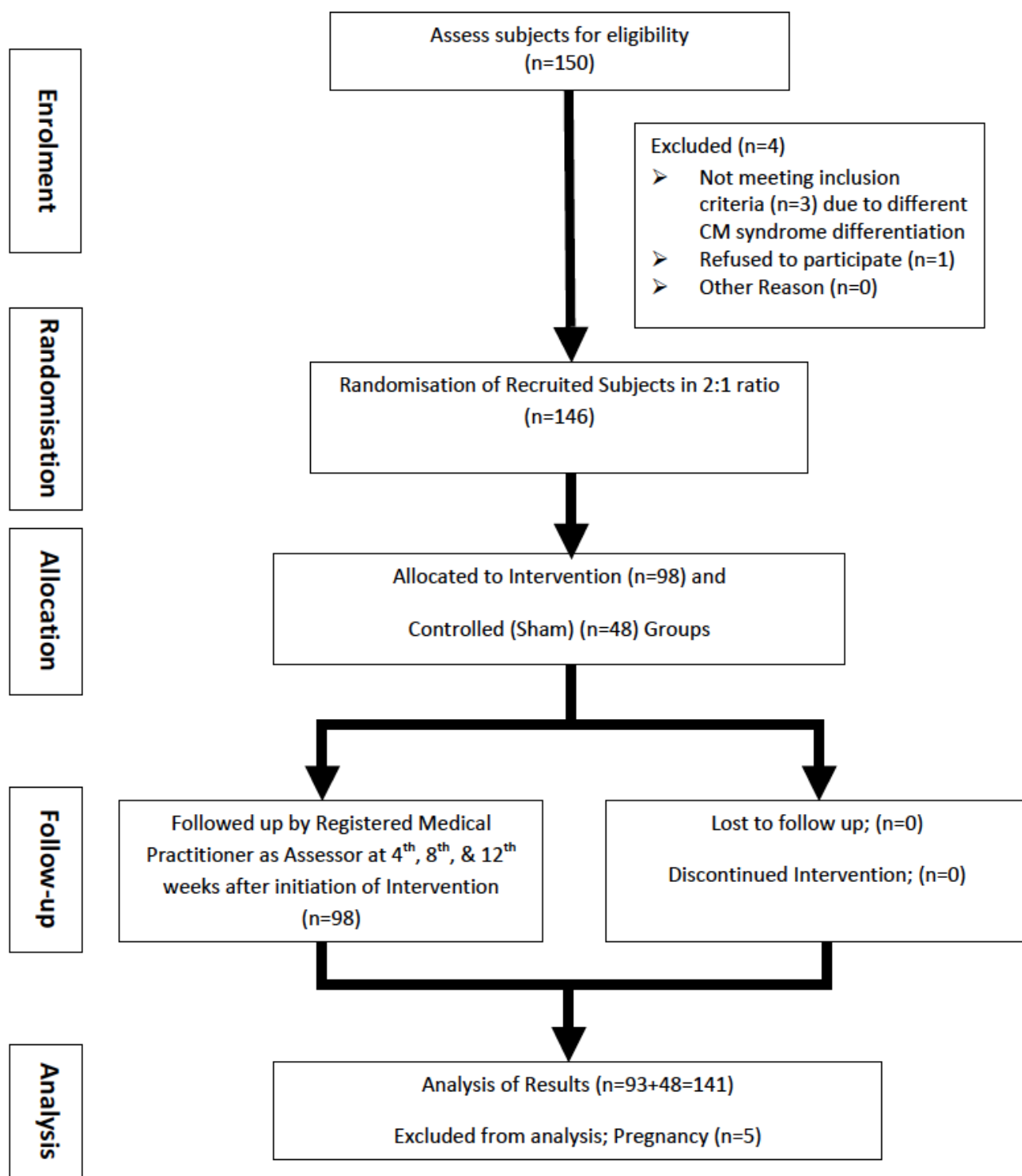
Figure 1: Flow Chart of Randomised Trial. (*Adapted & Modified from CONSORT guideline [54]*).

and 3 months follow up period after the completion of intervention. Specifically, the 3 months intervention period involved the weekly acupuncture (real or sham) intervention. This study was carried out in the period of Jan 2008 – May 2009. This study was approved by the Human Research Ethics Committee (HREC 07289) of the University of New South Wales on 29 Jan 2008 and was also registered with WHO trial register through Australian & New Zealand Clinical Trial Registry (ANZCTR) with Trial ID No. ACTRN12609000073202.

For the randomization of the sample, computer software "Randlist" was utilized for the sequence of the

participants that will undergo the prescribed treatment. A ratio of 2:1 (intervention: control) was employed in the distribution of the selected participants. Allocation concealing of participants were done through the use of pre-sealed identical envelopes. 2:1 allocation was employed in this study as to increase the recruitment rate and reduce trial time, which is often a problem in CM research. As Adam [7] and Avins [8] pointed out, "*timely recruitment is critical to the successful performance of any clinical trial. Failure to achieve recruitment goals can compromise statistical power or lead to an extension of the study time period and budget overruns*". This study ran over a seven months period which included 3 months intervention period, a higher dropout rate was anticipated. A higher recruitment rate into treatment may overcome this problem [9]. Furthermore, *"higher allocation to experimental treatment also has the advantage of increasing the likelihood of observing rare adverse events,"* in particularly when non properly designed acupuncture RCT was performed in this area [10].

Details of Acupuncture Intervention

The application of acupuncture to all participants and across the two groups was made sure to be uniform from one participant to another. All acupuncture sessions were performed by two study acupuncturists ie., one study acupunturist in China and one in Australia.

Sham Body Acupuncture

In this study, the sham acupuncture points selected were located approximately 10 mm outside the classical point and outside of the meridian, in order to ensure that the sham therapeutic effect was not increased due to the inadvertent stimulation of a distal part of the route of the meridian. In order to minimize the therapeutic effect of the sham treatment, superficial needling methods were used in this trial. The acupuncture needle itself has only inserted superficially enough on the skin to hold it in a desired angle.

Blinding at Needling

Moreover, it must be noted that the application of the acupuncture treatment was done on the participants with a curtain being set up at participant's chest level in order to block participant's view of the acupuncture needle application. So that the participant could not directly visualise the acupuncture needles and did not know whether she was receiving real or sham acupuncture intervention.

Needle Instrument Selection

Under this trial, thin (0.30 mm) needles were used as they provide less stimulation when compared to large diameter needles [11].

Needling Technique

In the real acupuncture group, once the arrival of Qi was achieved after needling, tonifying manoeuvre (clockwise insertion) was chosen. This technique has the effect of regulating Qi circulation in the related meridian and organ [11]. The needle was evenly rotated, thrusted and lifted [11]. During the 20 minutes needle retention, all needles had the scraping needling technique applied every 10 minutes. This is an acupuncture needling technique that uses the fingernail to scrape the handle of the needle in order to maintain Qi stimulation [11]. Nil stimulation was applied to the needle in the sham acupuncture group. In summary, the sham acupuncture effect can be minimized through careful selection of the sham needling points and instrument, depth of needling and the appropriate needling techniques.

Acupuncture Prescription

From our previous pilot study, we realise that most of the women with PCOS have signs with Kidney Yang deficiency pattern of Chinese medicine syndrome differentiation of PCOS. As such, in this RCT, only individual with Kidney Yang Deficiency pattern of PCOS and presented with complete amenorrhoea was recruited as it formed the majority of Chinese medicine syndrome differentiation in women with PCOS. Following groups were recruited into this study:

- Kidney Yang Deficiency

- Kidney Yang Deficiency with phlegm

- Kidney Yang Deficiency with phlegm and Blood stasis

All participants will receive a set of common points and those individuals with phlegm +/- blood stasis will also receive supplementary points. Table **1** summarises the acupuncture points used in this study.

Outcome Measures

For this study, there are two types of outcome measures that are under consideration. The primary outcome measure of this RCT was the return of menstruation from amenorrhea. The secondary measures were the changes in serum hormonal

Table 1:   Acupuncture Points Treatment Protocol for this Study

Body Points	Location
Common points	
Zhong Ji Ren 3中極	On the midline of the lower abdomen, 4 cun* inferior to the umbilicus and 1 cun superior to the pubic symphysis
Zi Gong M-CA-18子宮	Lies on the midline of the lower abdomen, 4 cun inferior to the umbilicus and 1 cun superior to the pubic symphysis. It is located one hand breadth lateral to this point
Sanyinjiao SP 6三陰交	On the medial side of the lower leg, 3 cun superior to the prominence of the medial malleolus, in a depression close to the medial crest of the tibia
Guanyuan Ren 4關元	On the midline of the lower abdomen, 3 cun inferior to the umbilicus and 2 cun superior to the pubic symphysis
Additional Points	
With Phlegm	
Zusanli ST 36足三里	Below the knee, 3 cun inferior to level immediately below the patella and medial to the patellar ligament, one finger breadth lateral to the anterior crest of the tibia
Feng Long ST 40豐隆	On the lower leg, midway between the tibiofemoral joint line (level with the popliteal crease) and the lateral malleolus, two finger breadths lateral to the anterior crest of the tibia
With Blood Stasis	
Tai Chong LV 3太冲	On the dorsum of the foot, between the first and second toes, run the finger along the interspace between the first and second metatarsal bones towards the ankle, into the pronounced depression before the junction of the bases of the first and second metatarsals
Guilai ST 29歸來	On the lower abdomen, 2 cun lateral to the midline and 4 cun inferior to the umbilicus, level with Zhongji Ren 3.
Xuehai SP 10血海	2 cun proximal to the superior border of the patella, in the tender depression on the bulge of the vastus medialis muscle

* "Cun" is a traditional Chinese unit of length. Its traditional measure is the width of a person's thumb at the knuckle, whereas the width of the two forefingers denotes 1.5 cun and the width of all fingers side-by-side is three cuns. In this sense it continues to be used to chart acupuncture points on the human body in various uses of traditional Chinese medicine. In modern China nowadays, 1 cun is equivalent to 3.33 cm.

profiles including LH, FSH concentration, LH: FSH Ratio, Progesterone concentration, Oestrogen concentration and Androgenic hormone concentration. Ovulation was not measured in this study.

The clinical parameters were measured through frequency of menstrual loss and timing as well as through the weight or body mass index of the patient. Symptomatic parameters included the menstrual loss at the conclusion of the post-intervention follow-up. Transvaginal ultrasound of ovaries was performed by an expert ultrasonographer at a set time (at Early Follicular Phase) in the patient's menstrual cycle, and the number and size of ovarian cysts recorded. Endocrine measures on the other hand were conducted at pre and post treatment during the early follicular phase. This included serum testosterone, sex hormone-binding globulin (SHBG), androstenedione, dehydroepiandrosterone sulphate (DHEAS), luteinising hormone (LH), estradiol, progesterone, 17-Hydroxyprogesterone, follicular stimulating hormone

(FSH), and LH:FSH ratio. All endocrinological testing were performed at the early follicular phase of the subject's menstrual cycle.

Statistical Analyses

All statistical analyses were performed using PASW (formerly SPSS) for Windows (PASW 18.0, SPSS Inc., Chicago, IL). All of the analyses were two-sided with a 5% alpha level. Demographic variables were summarized using the mean, standard deviation, and range for continuous scaled variables. Frequency and percentage were used for the categorical scaled variables.

Independent sample *t*-test was employed to both control and intervention group with regards to the differences of treatment outcomes. ANOVA was employed to detect any group difference between the placebo group and intervention group with regards to several pre and post treatment indicators.

RESULTS

Demographic data & Outcome Measures

Table **2** and **3** presents the demographic variables of the participants involved in each group. Table **2** presents the demographic information for the control group (real body acupuncture) while Table **3** presents the demographic information for the intervention group (sham body acupuncture). All statistical analysis of outcomes measures are illustrated in Tables **4**-**6**.

Table 2: Descriptive Data for Control Group

	N	Minimum	Maximum	Mean	Std. Deviation
Age	48	20.00	33.00	25.08	2.96
Height	48	1.41	1.68	1.54	0.05
Weight	48	39.00	75.00	54.06	9.52
BMI	48	18.30	32.02	22.84	4.43
Differences FSH	48	-2.20	3.80	0.42	1.38
Differences LH	48	-19.95	5.30	-2.34	4.53873
Difference Ratio LH:FSH	48	-4.64	2.31	-0.82	1.46
Difference E2	48	-95.00	47.00	-6.26	25.58
Difference Progesterone	48	-2.60	1.72	0.01	0.92
Difference FAI	48	-6.65	8.22	0.29	3.88
Difference Testosterone	48	-2.19	2.84	-0.11	1.20
Menstruation Days Pre Treatment	48	38.00	545.00	302.45	156.04
Menstruation Days Post Treatment	48	38.00	594.00	348.32	161.56
Difference Menstruation Days	48	-52.00	3.00	-45.87	12.42

Table 3:   Descriptive Data for Intervention Group

	N	Minimum	Maximum	Mean	Std. Deviation
Age	93	2.00	36.00	26.09	4.24
Height	93	1.44	1.76	1.59	0.04
Weight	93	41.00	107.80	57.44	12.46
BMI	93	15.63	41.08	22.61	4.73
Differences FSH	93	-3.86	3.12	-0.78	1.44
Differences LH	93	-3.43	18.28	5.09	4.77
Difference Ratio LH:FSH	93	-.82	3.53	1.23	0.98
Difference E2	93	-105.00	92.00	3.96	33.62
Difference Progesterone	93	-25.60	3.20	-3.37	7.03
Difference FAI	93	-3.44	87.04	24.20	19.34
Difference Testosterone	93	-2.00	50.00	29.63	13.37
Menstruation Days Pre Treatment	93	28.00	548.00	297.69	160.53
Menstruation Days Post Treatment	93	18.00	50.00	33.82	6.98
Difference Menstruation Days	93	-41.00	513.00	266.22	158.98

Table 4: Independent Samples t-Test for Control Group

	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
			Lower	Upper
Differences FSH	0.041	0.4210	0.0182	0.8238
Differences LH	0.001	-2.3418	-3.6598	-1.0240
Difference Ratio LH:FSH	0.000	-0.8183	-1.2440	-0.3926
Difference E2	0.097	-6.2627	-13.6924	1.1669
Difference Progesterone	0.931	0.0116	-0.2571	0.2805
Difference Prolactin	0.608	0.2895	-0.8381	1.4173
Difference FAI	0.000	-22.0552	-27.1127	-16.9977
Difference Testosterone	0.271	-0.3901	1.0953	0.3150
Difference Menstruation Days	0.001	0.3422	0.0032	14.334

Table 5: Independent Samples t-Test for Intervention Group

	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
			Lower	Upper
Differences FSH	0.000	-0.6392	-0.9792	-0.3745
Differences LH	0.000	4.3408	3.8646	5.0283
Difference Ratio LH:FSH	0.000	1.0338	0.9505	1.1707
Difference E2	0.321	2.4839	-3.8580	8.2941
Difference Progesterone	0.000	-3.4735	-5.2733	-2.0773
Difference Prolactin	0.432	-0.0961	-1.2879	0.9618
Difference FAI	0.000	20.7648	19.0812	23.5228
Difference Testosterone	0.543	0.0639	-0.2895	0.3833
Difference Menstruation Days	0.000	263.1931	229.1348	297.2516

Table 6:   ANOVA for Differences in between Control and Intervention Groups

Comparison Item	P value
Difference in Menstrual Days pre treatment	<0.0001
Difference in Menstrual Days post treatment	<0.0001
Difference in LH:FSH pre treatment	<0.0001
Difference in LH:FSH post treatment	0.0009
Difference in FAI pre treatment	<0.0001
Difference in FAI post treatment	<0.0001
Difference in Progesterone pre treatment	<0.0001
Difference in Progesterone post treatment	0.0001
Difference in Estradiol pre treatment	<0.0001
Difference in Estradiol post treatment	<0.0001
Difference in Prolactin pre treatment	<0.0001
Difference in Prolactin post treatment	<0.0001

Interpretation of Results

Based on the literature review [5], this is the first known randomised PCOS crossover single blind sham controlled body acupuncture trial that fully adheres to the traditional chinese medicine (CM) syndrome differentiation and at the same time employs a strict trial methodological protocol. The results from this trial demonstrate that acupuncture is effective in the menstrual management of PCOS. Real acupuncture treatment demonstrated statistically significantly better outcome than sham acupuncture treatment for the hormonal outcome measures of FSH, LH, LH: FSH ratio, Progesterone, and FAI. Therefore, the null hypothesis that real acupuncture treatment for PCOS based on traditional CM diagnosis and treatment procedures of no value is rejected.

Demographic Results

Altogether there are 48 women with PCOS recruited in the control group while 98 were recruited in the intervention group. In terms of the demographic data among the control and interventional groups, there are no significant difference between their age, height, weight and BMI. The mean age of the participants are 25.08 years old in the control group and 26.09 years old in the interventional group. The mean height of the participants is 1.52m and 1.59m respectively for the control and interventional group. The mean weight (and mean BMI) of the participant is 54.06 Kg (22.84) and 57.44 Kg (22.61) for the control and interventional group respectively.

Menstrual Days Outcome Measures

The mean inter-menstrual days in the control group is 302.45 day and 348.32 days pre and post treatment respectively (p=0.001). In the interventional group, the mean inter-menstrual days are 297.69 and 33.82 days pre and post treatment respectively (p=0.000). In between groups' analysis, high significance was noted for the difference in menstrual days before and after treatment. There are 5 pregnancy recorded in the interventional group at follow ups so they were excluded from the study. There are 10 out of 98 participants who remained to have amenorrhea while the rest (88/98; 89.79%) have menstruation return on with a mean days of 33.82 for 3 months at the follow up visit.

Hormonal Outcome Measures

In the control group before and after the sham acupuncture treatment, there are five treatment outcomes being found to have a significant difference among the FSH, LH, LH: FSH ratio, FAI, difference in menstruation days. All these treatments outcomes were said to have significant differences because its significance value is less than the alpha level of 0.05. On the other hand, in the interventional group, it can be seen that six treatment outcomes were found to have a highly significant differences (p<0.0001) pre and post real acupuncture intervention. These six treatments outcomes are FSH, LH, LH: FSH ratio, Progesterone, FAI, and menstruation days. There are no statistical significances (p>0.05) in testosterone, prolactin and estradiol in both control and interventional groups, despite both testosterone and estradiol have decreased in their mean level in the intervention group at the post treatment period. In between groups' analysis (control and interventional groups), high significance was noted among the difference in LH:FSH, FAI, Progesterone, estradiol and Prolactin levels before and after treatment.

Overall Findings

In the control group, FSH (p<0.041) appeared to be decreased marginally while LH (p<0.001), LH:FSH (p<0.000), E2 (p<0.097), FAI (p<0.000), Progesterone (p<0.931) and Testosterone (p<0.271) were recorded in higher level in the post treatment period as compared to those before treatment. There is no menstrual pattern change in the control group pre and post treatment. In the interventional group, FSH (p<0.000) and progesterone (p<0.000) level were higher in the post treatment period whereas LH (p<0.000), LH:FSH (p<0.000), FAI (p<0.000), E2 (p<0.321), and testosterone (p<0.543) were reduced in level. The menstruation days has decreased highly significantly from 297.69 days before treatment to 33.82 days after treatment.

DISCUSSION

Outcome Measures

The primary aim of this trial was to evaluate the whether acupuncture has a role in restoring regular menstrual pattern among women with PCOS. A subjective clinical assessment was used to measure the clinical outcome regarding to their menstruation pattern. Furthermore, an attempt was made to minimise, in the control group, the potential therapeutic effect possible for sham acupuncture. The results from this study demonstrated the usefulness of acupuncture treatment in the menstrual pattern management of

PCOS. This study was not aimed to elicit whether acupuncture induce ovulation or not since the investigators believe it was not fair to attempt that area prior to realising whether acupuncture does work on menstruation pattern regulation or not in PCOS.

Design Method

The type of research undertaken determined the selection for the blind study design [12]. Acupuncture is a technique based on a manipulative modality requiring individualized point selection and skin penetration [13] and stimulation [11]. Therefore, it would be impossible to meet the requirements of a double blind design [13] ie., both the acupuncturist and the subject being blinded completely. The best available option for an acupuncture research would therefore be a single blind design, in which the subjects were not told whether they were receiving a real treatment or sham treatment.

The advantages of a crossover design included it is an accepted method for clinical trials with fewer subjects [12, 14]; it is a cost efficient design; it is useful when the time frame of the treatment for a condition/disease is limited [14]; and it is more ethically acceptable as all subjects receive a real treatment.

On the other hand, there are conflicting reports stating that it is inappropriate to use crossover design. It is because acupuncture has a slow onset of action and produces long-term effects [13].

Sham Acupuncture

Currently there are no agreed gold standard or international consensus on how sham acupuncture should be performed. A variety of aspects have been addressed in this study in order to minimize the level of effects produced by the sham acupuncture, these include the location of the sham points selected, depth of needling, needle instrument selection and needling technique.

Location of the Sham Points

Sham acupuncture commonly used non-classical points or points outside the classically described meridian [15]. These sham acupuncture points have been chosen at 5 to 10 mm outside the classical points or classical meridian.

Depth of Needling

The depth of needling is a fine balance. On one hand, superficial needling methods were recommended [15] to minimize the therapeutic effect of the sham acupuncture. On the other hand, the needles required certain depth so that they will hold at a desired angle.

Needle Instrument Selection

The selection of needle thickness is another factor that can be adjusted to minimize the therapeutic effect of the sham acupuncture. Thin needles (0.26 mm) were used rather than large diameter needles, in order to have less stimulation (Cheng 1998), as well as able to minimize the depth of needling while still maintaining the needles at a desirable angle.

Needling Technique

Needling technique is fundamental in controlling the level of needle stimulation, which will provoke the neurophysiological effect [16] and directly influence the treatment outcome [11]. Accordingly, this study used minimal needle stimulation to sham points in order to reduce the therapeutic effects of sham acupuncture.

In conclusion, the sham acupuncture effect can be lessened through careful selection of the sham needling points' location, depth of needling, the thickness of needles and appropriate needling technique.

Strengths and Limitations

This study is primarily aimed to elicit whether acupuncture can assist in return of menstruation for women with PCOS and has complete amenorrhoea. As this is the initial aim of the study, ovulation was not chosen to be the outcome measure for this RCT. This also becomes one of the limitations for this study as we cannot definitely answer whether acupuncture can induce ovulation for lady with PCOS, although improvements were noted in the LH:FSH ratio and progesterone level. It is important to note that menstruation return may not necessary to protect the uterus from hyperplasia. Other limitation with this study was lack of measurement of hirsutism, which might not have been informative as this is not the primary aim of this study.

The strengths of this study are the RCT design, with its prospective data collection and single blinding of the intervention. Furthermore this is the first world properly designed RCT in this area that have employed the Chinese medicine syndrome differentiation while planning for the acupuncture points intervention protocol. Every single disease in western medicine has

its own Chinese medicine (CM) syndromes differentations. According to the CM theory, different syndrome would warrant different treatment protocol. Within one disease or diagnosis, there are several possible syndrome differentations from CM prospective. As such, this study was able to incorporate this into the treatment protocol and tested its effectiveness by integrating both western medicine (WM) and CM prospectives. For PCOS, the key pathogenesis is the deficiencies of Kidney functions with possible retention of phlegm and/or blood stasis. Combining these two factors, Kidney fails to disperse Qi and blood, which results in the key signs and symptoms of PCOS. Therefore, CM treatment should focus on the restoration of the Kidney yang deficiencies while simultaneously disperse phlegm and promote qi and blood flow. We believe it is important to incorporate CM theroy and syndrome differentiation while developing any acupuncture RCT invervention protocol, or otherwise it can easily lead to design and procedural bias, as well as type II and III errors.

This study was also successful in attaining the target sample size for adequate statistical power for the primary and secondary outcome measures. This study has the largest sample size of any prospective acupuncture study yet published for women with PCOS. All the hormonal assessments occurred in the early follicular phase, which is the time when inter-individual differences would be minimized, thus reducing measurement bias. From the real-world acupuncture practice point of view, this study allowed individualisation of acupuncture protocol. Therefore the interpretation of the results was not limited to the single acupuncture protocol.

Comparison with Other Related Studies

Oligo/amenorrhoea that is usually associated with anovulatory menstrual cycles and sub-fertility is included in the clinical presentation of polycystic ovary syndrome. Patients with PCOS have difficulty with conception and require a hierarchical structure of treatments to bring about ovulation. Patients are advised to lose weight when an increased BMI is detected as a preliminary recommendation. Oral anti oestrogen treatment in the form of medications such as clomiphene citrate is usually considered a first line of medical treatment. However, Hammond [17] suggested that about 15% of the patients remain in the anovulatory condition in spite of being provided with such a treatment. Furthermore, Zarte [18] and Raj [19] reported that only about less than fifty per cent of

women with "PCOS on anti-oestrogen treatment" were able to conceive. In such a situation the conventional treatment recommended is the administration of exogenous gonadotrophin preparations according to ESHRE [20] or the more modern method in the form of laparoscopic ovarian drilling according to Gadir [21]. As a general observation, Balen [22] reported that women with PCOS have been found to have experienced less success with gonadotrophin stimulation compared to women who have hypogonadotrophic amenorrhoea. Farhi [23] suggested that gonadotrophin stimulation has the possibility of affecting overstimulation (follicular development leading to cycle cancellation), ovarian hyper-stimulation syndrome and multiple pregnancies because the ovaries of women with PCOS are known to be highly sensitive to such stimulation when compared to others with unaffected ovaries. Researchers have found that such problems have the effect of increasing androgen and luteinising hormone (LH) levels, which are common in PCOS [24, 25]. It has been suggested theoretically speaking, that for cases where PCOS is related to hyper secretion of LH, purified follicle stimulating hormone or u-FSH concoctions are more effective compared to human menopausal gonadotrophin (hMG) concoctions (with FSH & LH). Yet, it is still not very clear whether this positive advantage can be successfully replicated in practice. Tartagani [26] suggested that by using gonadotrophin releasing hormone analogues one could improve the success rates of the outcomes of such treatments, while also being able to reduce unnecessary complications such as Ovarian Hyperstimulation Syndrome (OHS) and also multiple pregnancies. Gonadotrophin treatments are either channelled through intramuscular or subcutaneous routes or by various stimulation protocols and regimens as necessary (step up or standard) in order to improve its overall efficacy. However Greenblatt [27] pointed out that there are risks and morbidity associated with the laparoscopic procedure under general anaesthetic related, post-operative adhesion. Furthermore it is reported that there is also a long-term risk of premature ovarian failure. While ovarian drilling appears to normalize the endocrine environment, its long-term effect on the ovary is unknown. Recent findings suggest recombinant FSH (r-FSH) concoctions have been used successfully in medical practice. However, it is not clear whether the increased bioactivity of r-FSH discovered in recent IVF trials is conducive for bringing about ovulation. Furthermore, it is also not clear whether using r-FSH is likely to lead to an avoidance of high dosage and a longer time period. All things

considered one cannot ignore the cost component of r-FSH as r-FSH has been found to be more expensive than u-FSH. Therefore, there is not much empirical evidence to suggest that r-FSH is better than u-FSH for women with PCOS.

There is a lack of evidence in the western scientific literature to clarify the mechanism of acupuncture for PCOS. Despite the fact that the understanding of acupuncture is based on ancient medical practices dictated by Traditional Chinese Medicine (TCM), modern perspectives based on neuroendocrinal sciences have shed new light on the practice. As such, Mayer [28] was one of the first to report that acupuncture analgesia can be brought about through endorphin production and negated by narcotic antagonist naloxone. Studies by Ku [29], Petti [30] and Ulett [31] also suggested acupuncture related effects are channelled through the nervous system with specific references to ß-endorphin and other neuro-peptides.

The formation of increased levels of β-endorphin: during acupuncture treatment had been explained by Petti [30]. According to the researchers Faccinetti [31], Gallineli [32] and DeBold [33], β-Endorphin is a derivative of the precursor protein, pro-opiomelanocortin that is available in huge quantities in the neuronal cells of the arcuate nucleus of the hypothalamus, pituitary, medulla and also the peripheral tissues such as the intestines and ovaries. The formation takes place when Pro-opiomelanocortin forks to form adrenocorticotropic hormone and β-lipoprotein which then again fork to form β-endorphin. Aleem [34,35] showed that immunoreactive β-endorphin was present in "follicular fluids" in both normal and polycystic ovaries.

Aleem *et al.* [34] reported that the endogenous opioid peptides action on GnRh secretion was responsible for bringing about gonadotrophin secretion and menstrual cycle. The hypothalamic β-endorphin centre and the GnRH pulse generator, in fact, are both located within the arcuate nucleus. Quigley [36] was one of the first to report that LH secretion that was limited by opioids in hyperprolactinaemia patients suffering from pituitary microadenomas. Other scientists such as Ching [37] and Orstead and Spics [38] demonstrated that opioid peptides inhibit GnRH release in animals such as rats and rabbits. Kalra [39] and Pau & Spies [40] further reviewed the role played by neuropeptides such as β-endorphin in limiting GnRH release, while Rossmanith [41] showed that opioid

peptides had a role in bringing about the commencement of the mid cycle LH surge in women with normal menstrual cycles. Petragglia [42] found that the level of β-endorphin was higher in ovarian follicular fluid compared to the circulating fluid when measured in healthy ovulating women where the highest levels were found in the pre-ovulatory follicle.

It can be inferred that acupuncture results in ovulation and fertility as a consequence of studies that have shown that these treatments have an influence of increased β-endorphin levels affecting the release of GnRH and also the menstrual cycle. Studies that have been conducted in animals by Yang [43] have suggested that acupuncture treatment was successful in regularizing GnRh release and peripheral gonadotrophin levels.

Researchers such as Aso [44], Yu [45], Mo [46] and Cai [47] had shown that acupuncture positively affected the plasma levels of FSH, LH, oestrogen and progesterone. Cai [47] showed that acupuncture could be used as a surrogate for hCG in bringing about ovulation; Chen and Yu [48] demonstrated the regularization of hypothalamic-pituitary-ovarian axis, while Chen [49] showed that acupuncture treatment was successful in bringing about responses in six out of thirteen anovulatory cycles.

In another experiment, Stener-Victorin [50] reported that there was an improvement in ovulation cycles from 15% to 66% over a three month period between pre-treatment phase and post treatment phases when the method of electro-acupuncture was used for ovulation induction in twenty four oligo/amenorrhoeic women suffering from polycystic ovarian syndrome or PCOS. The experiments showed that the patients who had responded to the treatment had a lower body mass index, waist to hip circumference ratio, serum testosterone, sex hormone globulin ratio and serum basal insulin levels, suggesting that acupuncture could be used as an alternative to pharmacological ovulation induction.

Another 2 studies [51, 52] used electro-acupuncture RCT also showed that low frequency electro-acupuncture seemed to improve hyperandrogenism and menstural frequency than no intervention in women with PCOS [51], as well as having significant changes of LH, FSH level and also higher ovluation frequency [52]. On the other hand, Pastore [53] performed a double blind, randomised sham controlled clinical trial in women with PCOS over 5 months period.

They were not able to detect any different between true and sham acupuncture on serum LH and FSH level, as well as ovulation before and after intervention. Acupuncture points used in their study include Bladder 23, Bladder 28, Spleen 6, Spleen 9, Pericardium 6, Triple energizer 5, and Governor vessel 20. On the contrary, our study have found significant changes in its hormonal assessment and menstruation rate before and after acupuncture intervention. One of the possible reasons may be related to the fact that our study has developed the acupuncture intervention protocol based on its CM syndrome differentiation rather than an universal set of points.

CONCLUSION

From this study, we can conclude that it seems like acupuncture has a significant effect towards the changes of FSH, LH, LH: FSH ratio, FAI, and menstruation rate in women with PCOS. The discrepancy of the conclusion between our study and another body acupuncture RCT [53] may be related to fact CM syndrome differentiation on acupuncture intervention protocol development was used in this study rather than using an universal set of acupuncture points for all women with PCOS. However, this will need to be validated in further large scale RCT before a definitive conclusion can be drawn.

ACKNOWLEDGEMENT

REFERENCES

[1] Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, *et al.* Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril, 2012. 97(1): p. 28-38 e25.

[2] Escobar-Morreale HF, Carmina E, Dewailly D, Gambineri A, Kelestimur F, *et al.* Epidemiology, diagnosis and management of hirsutism: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update, 2012. 18(2): p. 146-70. http://dx.doi.org/10.1093/humupd/dmr042

[3] Thessaloniki ESHRE/ASRM-Sponsored PCOS consensus workshop group. Consensus on infertility treatment related to polycystic ovary syndrome. Fertil Steril, 2008. 89(3): p. 505-22. http://dx.doi.org/10.1016/j.fertnstert.2007.09.041

[4] Stener-Victorin E, Jedel E, Mannera L. Acupuncture in Polycystic Ovary Syndrome: Current Experimental and Clinical Evidence. Journal of Neuroendocrinology 2008; 20:290-298. http://dx.doi.org/10.1111/j.1365-2826.2007.01634.x

[5] Lim DCE, Chen W, Cheng NL, Xue CC, Wong FWS, O Sullivan AJ, Liu JP. Cochrane Database of Systematic Reviews 10(8):1-18 2011

[6] Rotterdam ESHERE/ASRM sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS). Human Reproduction 2004;19(1):41-7. http://dx.doi.org/10.1093/humrep/deh098

[7] Adams J, Silverman M, Musa D, Peele P. Recruiting older adults for clinical trials. Control Clin Trials. 1997;1:14–26. http://dx.doi.org/10.1016/S0197-2456(96)00132-8

[8] Avins AL. Can unequal be more fair? Ethics, subject allocation, and randomised clinical trials. J Medical Ethics. 1998;24:401–08. http://dx.doi.org/10.1136/jme.24.6.401

[9] Aisen PS, Schafer KA, Grundman M, *et al.* Effects of rofecoxib or naproxen *vs*. placebo on Alzheimer disease progression – A randomized controlled trial. JAMA. 2003;21:2819–26. http://dx.doi.org/10.1001/jama.289.21.2819

[10] Vazdolska R, Sano M, Aisen P, Edland SD. The net effect of alternative allocation ratios on recruitment time and trial cost. Clinical Trials. 2009;6(2):126-132. http://dx.doi.org/10.1177/1740774509103485

[11] Cheng XN. Chinese Acupuncture and Moxibustion. Beijing: Foreign Languages Press; 1998.

[12] Gross-Portney L, Watkins MP. Foundations of Clinical Research: Applications to Practice. Appleton & Lange: Norwalk. 1993

[13] World Health Organisation, Regional Office for the Western Pacific. Guidelines for clinical research on acupuncture. WHO Regional Publication. Western Pacific series No. 15. 1995

[14] Ratain JS, Hochberg MC. Clinical trials: A guide to understanding methodology and interpreting results. Arthr Rheum 1990;33(1):131-9. http://dx.doi.org/10.1002/art.1780330118

[15] Vincent C, Richardson P. The evaluation of therapeutic acupuncture concepts and methods. Pain. 1986;24:1-13. http://dx.doi.org/10.1016/0304-3959(86)90022-9

[16] Baldry PE. Acupuncture, Trigger Points and Musculoskeletal Pain, Churchill Livingston. 1993

[17] Hammond M, Halme J, Talbert L. Factors affecting the pregnancy rate in clomiphene citrate induction of ovulation. Obstet Gynecol 1983;62:196–202

[18] Zarte A, Herdmandez-Ayup S, Rios-Montiel A. Treatment of anovulation in the Stein-Leventhal syndrome. Analysis of ninety cases. Fertility & Sterility 1971;22:188-93

[19] Raj S, Thompson I, Berger M, Taymor M. Clinical aspects of polycystic ovary syndrome. Obstetrics and Gynecology 1977;49:552-6

[20] ESHRE Capri Workshop. Female infertility: treatment options for complicated cases. Human Reproduction 1997;12:1191-6. http://dx.doi.org/10.1093/humrep/12.6.1191

[21] Gadir AA, Mowafi RS, Alnaser HMI, Alrashid AH, Shaw RW. Ovarian electrocautery versus hMG and pure FSH therapy in the treatment of patients with polycystic ovarian disease. Clinical Endocrinology 1990;33:585-92. http://dx.doi.org/10.1111/j.1365-2265.1990.tb03896.x

[22] Balen A. The pathophysiology of polycystic ovarian syndrome: trying to understand PCOS and its endocrinology. Best Practice and Research Clinical Obstetrics and Gynaecology. 2004;18(5):685-706. http://dx.doi.org/10.1016/j.bpobgyn.2004.05.004

[23] Farhi J, West C, Patel A, Jacobs H. Treatment of anovulatory infertility: the problem of multiple pregnancy. Human Reproduction 1996;11:429-34.
http://dx.doi.org/10.1093/HUMREP/11.2.429

[24] Homburg R. Involvement of growth factors in the pathophysiology of polycystic ovary syndrome. Gynecol Endocrinol 1998;12:391-397.
http://dx.doi.org/10.3109/09513599809012841

[25] Regan L, Owen E, Jacobs H. Hypersecretion of luteinising hormone, infertility and miscarriage. Lancet 1990;336:141-4.
http://dx.doi.org/10.1016/0140-6736(90)92765-A

[26] Tartagni M, Diaferia A, Nicastri P, Loizzi P. Induction of multifollicular growth in patients non responding to clomiphene citrate and gonadotrophins or gonadotrophins only. Clinical and Experimental Obstetrics and Gynecology 1995;22:315-20

[27] Greenblatt E, Casper R. Adhesion formation after laparoscopic ovarian cautery for polycystic ovarian syndrome: lack of correlation with pregnancy rate. Fertility & Sterility 1993;60:766-70

[28] Mayer DJ, Price DD, Rafil A. Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. Brain Research 1977;121:368-72.
http://dx.doi.org/10.1016/0006-8993(77)90161-5

[29] Ku Y, Chang Y. Beta-endorphin and GABA-mediated depressor effect of specific electroacupuncture surpasses pressor response of emotional circuit. Peptides 2001;22:1465–70.
http://dx.doi.org/10.1016/S0196-9781(01)00467-3

[30] Petti F, Bangrazi A, Liguori A, Reale G, Ippoliti F. Effects of acupuncture on immune response related to opioid-like peptides. Journal of Traditional Chinese Medicine 1998;18:55-63.

[31] Ulett GA, Han S, Han JS. Electroacupuncture: mechanisms and clinical application. Biological Psychiatry 1998;44:129-38.
http://dx.doi.org/10.1016/S0006-3223(97)00394-6

[32] Gallinelli A, Garuti G, Matteo ML, Genazzani AR, Facchinetti F. Expression of proopiomelanocortin gene in human ovarian tissue. Hum Reprod 1995;10:1085–9

[33] DeBold CD, Menefee JK, Nicholson WE, Orth DN. Pro-opiomelanocortin gene is expressed in many normal human tissues and in tumours not associated with ectopic adrenocorticotropin syndrome. Mol Endocrinol 1988; 2:862–70.
http://dx.doi.org/10.1210/mend-2-9-862

[34] Aleem FA, Eltabbakh GH, Omar RA, Couthren AL. Ovarian follicular fluid beta-endorphin levels in normal and polycystic ovaries. American Journal of Obstetrics and Gynecology 1987;156:1197-1200.
http://dx.doi.org/10.1016/0002-9378(87)90143-8

[35] Aleem FA, Omar RA, Eltabbakh GH. Immunoreative beta-endorphin in human ovaries. Fertil Steril 1986;45:507–11.

[36] Quigley ME, Sheeham KL, Casper RF, Yen SSC. Evidence for an increased opioid inhibition of luteinizing hormone secretion in hyperprolactinemic patients with pituitary microadenoma. J Clin Endocrinol Metabol 1980;50:427–46.
http://dx.doi.org/10.1210/jcem-50-3-427.

[37] Ching HL, Burke V, Stucke BGA. Quality of life and psychological morbidity in women with polycystic ovary syndrome: body mass index, age and the provision of patient information are significant modifiers. Clinical Endocrinology, 2007; 66(3): 373-379.
http://dx.doi.org/10.1111/j.1365-2265.2007.02742.x

[38] Orstead KM, Spics HG. Inhibition of hypothalamic gonadotropin releasing hormone release by endogenous opioid peptides in the female rabbit. Neuroendocrinology 1987;46:14-23.
http://dx.doi.org/10.1159/000124791

[39] Kalra SP, Horvath T, Naftolin F, Xu B, Pu S, Kalra PS. The interactive language of the hypothalamus for the gonadotropin releasing hormone (GNRH) system. J Neuroendocrinol 1997;9:569–76.
http://dx.doi.org/10.1046/j.1365-2826.1997.00619.x

[40] Pau KY, Spies HG. Neuroendocrine signals in the regulation of gonadotropin-releasing hormone secretion. Chin J Physiol 1997;40:181–96.

[41] Rossmanith WG, Mortola JF, Yen SSC. Role of endogenous opioid peptides in the initiation of the mid-cycle luteinizing hormone surge in normal cycling women. J Clin Endocrinol Metab 1988;67:695–700.
http://dx.doi.org/10.1210/jcem-67-4-695

[42] Petraglia F, DiMeo G, Storchi R, Segre A, Facchinette F, Szalay S. Proopiomelanocortin-related peptides and methionine enkephalin in human follicular fluid: changes during the menstrual cycle. American Journal of Obstetrics and Gynecology 1987;157:142-6.
http://dx.doi.org/10.1016/S0002-9378(87)80366-6

[43] Yang SP, Yu J, He L. Release of gonadotropin-releasing hormone (GnRH) from the medio-basal hypothalamus induced by electroacupuncture in conscious female rabbits. Acupunct Electrother Res 1994; 19:19–27

[44] Aso T, Motohashi T, Murata M, Nishimura T, Kakizaki K. The influence of acupuncture stimulation on plasma levels of LH, FSH, progesterone and estradiol in normally ovulating women. American Journal of Chinese Medicine 1976;4:391-401.
http://dx.doi.org/10.1142/S0192415X76000494

[45] Yu J, Zheng HM, Ping SM. Changes in serum FSH, LH and ovarian follicular growth during electroacupuncture for induction of ovulation (Chinese). Chung Hsi I Chieh Ho Tsa Chih 1989;9:199–202

[46] Mo X, Li D, Pu Y, Xi G, Le X, Fu Z. Clinical studies on the mechanism of acupuncture stimulation of ovulation. Journal of Traditional Chinese Medicine 1993;13:115-9.

[47] Cai X. Substitution of acupuncture for human chorionic gonadotropin in ovulation induction. Journal of Traditional Chinese Medicine 1997;17:19-21.

[48] Chen BY, Yu J. Relationship between blood radioimmunoreactive beta-endorphin and hand skin temperature during the electro-acupuncture induction of ovulation. Acupuncture & Electro-therapeutics Research 1991;16:1-5.

[49] Chen BY. Acupuncture normalizes dysfunction of hypothalamic-pituitary-ovarian axis. Acupunct Electrother Res 1997;22:97–108.

[50] Stener-Victorin E, Waldenstrom U, Tagnfors U, *et al.* Effects of electro-acupuncture on anovulation in women with polycystic ovary syndrome. Acta Obstet Gynecol Scand, 2000; 79(3):180–8.
http://dx.doi.org/10.1080/j.1600-0412.2000.079003180.x

[51] Jedel E, Labrie F, Oden A, Holm G, Nilsson L, *et al.* Impact of electro-acupuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial. Am J Physiol Endocrinol Metal 2010; 300: E37-E45.
http://dx.doi.org/10.1152/ajpendo.00495.2010

[52] Johansson J, Redman L, Veldhuis PP, Sazonova A, Labrie F, *et al.* Acupuncture for ovulation induction in polycystic ovary syndrom: randomised controlled trial. Am J Physiol Endocrinol Metab 2013; 304: E934-E943.
http://dx.doi.org/10.1152/ajpendo.00039.2013

[53] Pastore LM, Williams CD, Jenkins J, Patrie JT. True and sham acupuncture produced similar frequency of ovulation and improved LH to FSH ratios in women with polycystic ovary syndrome. J Clin Endocrin Metab. 2011; 96(10); doi:10.1210/jc.2011-1126.
http://dx.doi.org/10.1210/jc.2011-1126

[54]   Moher, Schutz, Altman. The CONSORT Statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. Lancet 2001; 357: 1191–94.
http://dx.doi.org/10.1016/S0140-6736(00)04337-3