JoP Journal of Pharmacopuncture

Journal of Pharmacopuncture 2023;26(2):105-123
pISSN: 2093-6966 • eISSN: 2234-6856
https://doi.org/10.3831/KPI.2023.26.2.105

Review Article

A Review on Acupuncture Efficiency in Human Polycystic Ovary/Ovarian Syndrome

Natália M. de Oliveira[1,2]*, Jorge Machado[1,2,3], Lara Lopes[1,2], Maria Begoña Criado[2,4]

[1]ICBAS - Institute of Biomedical Sciences Abel Salazar, School of Medicine and Biomedical Sciences, University of Porto-UP, Porto, Portugal
[2]CBSin, Center of BioSciences in Integrative Health, Porto, Portugal
[3]LABIOMEP – Porto Biomechanics Laboratory, University of Porto-UP, Porto, Portugal
[4]TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, Gandra, Portugal

Received April 20, 2021
Reviewed March 14, 2022
Accepted February 28, 2023

***Corresponding Author**
Natália M. de Oliveira
ICBAS - Institute of Biomedical Sciences Abel Salazar, School of Medicine and Biomedical Sciences, University of Porto-UP, Rua de Jorge Viterbo Ferreira 120, Massarelos, Porto 4050-313, Portugal
Tel: +351-22-042-8304
E-mail: nmdeoliveira22@gmail.com

Objectives: Human Polycystic Ovary/Ovarian Syndrome (PCOS), a frequent endocrine pathophysiology among women of fertile age, is associated with woman's lifespan endocrine, metabolic and psychological implications. Long-term usage side effects of allopathic strategies and their low efficacy made complementary medicine treatments a relevant subject for these patients. The main aim of this work is to review the efficiency of the acupuncture treatment reported in the latest studies on literature regarding PCOS patients.
Methods: A comprehensive literature search, in English language, about the use of acupuncture on the management of PCOS, was conducted in October 2020 using EBSCO, Cochrane, PubMed, Medline, Embase, databases for randomised and non-randomised controlled trials between 2015 and 2020 (09/2015-10/2020), following the PRISMA protocol.
Results: This research led us to an analysis, according to PICOS, of six final papers selected among 178 in total. The articles addressed distinct aspects of the PCOS condition, diverse acupuncture techniques and different main and secondary outcomes in agreement to the respective main objective. According to this review, acupuncture seems to be useful for the treatment of this debilitating and chronic health condition which affects millions of women worldwide, many of them part of the workforce in their community.
Conclusion: Despite of the display of these positive results addressing acupuncture treatments to manage PCOS major symptoms - whether they are reproductive, metabolic, or mental health features - more research is urgent. Randomized, double blinded controlled trials with improved quality design and following STRICTA and/or CONSORT recommendations are necessary to engage acupuncture as a standardized and scientific validated technique in PCOS condition.

Keywords: acupuncture therapy, mental health, metabolic dysfunction, ovarian dysfunction, PCOS, TCM

INTRODUCTION

The Stein-Leventhal syndrome, known as Polycystic Ovary/Ovarian Syndrome (PCOS), is the most frequent endocrine pathophysiology among women of fertile age, displaying a prevalence between 5-15%, depending on the clinical diagnostic criteria adopted [1, 2]. The aetiology of this disorder is still unidentified, and its diagnostic is always made by exclusion of conditions that mimic the phenotypes [3]. A simplified current aetiology of the PCOS condition is attributed to a "two-hit" theory. A first hit such as congenital predisposition *in utero* interplayed by multiple factors – including increased anti-Müllerian hormone (AMH) concentrations, restriction in fetal nutrition, endocrine disruptors, and androgen excess – leads to

development of a PCOS-like phenotype in adulthood when the second hit happens. The second hit is the exposure to post-natal inflammatory factors such as obesity and insulin resistance [4]. Endocrine and metabolic implications of the syndrome reflect a high comorbidity [5] within three major aspects: hyperandrogenism [6-9] and reproductive issues [10-13], metabolic [14-30], and mental or emotional [31-39]. PCOS has also been strongly associated with alterations in the autonomic nervous system – specifically increased sympathetic nervous activity [40]. Due to these multiple health implications, many women with PCOS require long-lasting treatments which range from lifestyle modification to precise medical or surgical methods.

Distinct clinical approaches have been designed for PCOS management according to international guidelines [41-44]. Despite these efforts, women with PCOS exhibited disappointment regarding their clinical health management [44]. Furthermore, allopathic therapies have displayed multiple unpleasant secondary effects which motivate patients with PCOS to search for complementary medicines like Traditional Chinese Medicine (TCM). Among the different therapeutic approaches of TCM, acupuncture is the most known in occident, where it has had marked progress in the second half of the twentieth century [45]. Acupuncture treatment involves insertion of very thin metal needles into specific areas or acupoints of the body, which are important in regulation and restoration of vegetative function [46]. Different studies demonstrate several characteristics associated with these acupoints: correspondence to a dermatome pattern [45], various types of terminal nerves [47], reduced skin electric impedance, and the presence of neurovascular bundles [48].

Regarding the syndrome of Stein-Leventhal, acupuncture has been used as therapy or co-therapy to improve the comorbidity of symptoms affecting women with this condition. The effects of acupuncture on PCOS are related to sexual hormone regulation, improvement in ovulation and pregnancy rate, insulin resistance and lipid metabolism regulation as well as the rebalance of negative emotions. Diverse studies on the use of low frequency electroacupuncture (EA), alone or in combination with Western medicine or with exercise [49, 50] to modulate the sympathetic nervous system, reveal that acupuncture regulates the hypersecretion of androgens improving both reproductive and endocrine metabolic functional states [40]. The circulating and adipose tissue androgen concentrations in patients with PCOS are decreased by EA, suggesting that the effect of EA may be associated with decreased levels of hemoglobin A1c (HbA1c) [51]. Animal studies also indicate that the efficacy of acupuncture may be related to the inhibitory effect on overexpression of androgen receptor and connexin 43 [52]. Low-frequency EA also reduced serum testosterone in rats with PCOS, which may be mediated by central opioid receptors such as Oprk1 and Oprm1 in the hypothalamic arcuate nucleus [53]. Acupuncture has been also used, alone or in combination with clomiphene, to ameliorate the disorder of subfertility in PCOS patients [54, 55]. With respect to psychological balance, acupuncture studies have been designed to relieve anxiety and depression through the regulation of circulating β-endorphins and androgens in patients with PCOS [56]. Electroacupuncture interventions inhibited the hyperactivity of the sympathetic nervous system in PCOS rats, likely related to the inhibitory effects of EA on nerve growth factor (NGF) concentrations in ovaries [57]. Electroacupuncture also prevents an increase in p75 neurotrophin receptor (p75NTR) expression – probably by normalizing the sympathetic ovarian response to NGF action [58] – and p75NTR is crucial in the sympathetic nervous system during development [59].

Acupuncture decreases cortisol concentrations and regulates central and peripheral β-endorphins production and secretion [60]. Considering that acupuncture has a potential effect on β-endorphins, which can impact gonadotropin-releasing hormone (GnRH) secretion and concentrations, it is postulated that acupuncture may play an important role in improving ovulation induction and fertility. Furthermore, EA proved efficacious in improving oocyte quality and embryonic development potential in infertile patients with PCOS. Upregulation of the IRS-1/PI3K/GLUT4 signaling pathway appears to be involved in the effect of EA [61]. In addition, EA improved abnormal follicular development in women with PCOS by arresting the overexpression of AMH and enhancing the expression of $P450_{arom}$ [62]. Plus, EA amended ovulation disorders by downregulating LncMEG3 expression, inhibiting the PI3K/AKT/mTOR pathway, and reducing granulosa cell autophagy in animal studies [63]. EA improved follicular arrest in PCOS rats by reduction of the overexpression of AMH to normalize follicle-stimulating hormone (FSH) and AMH imbalance in granulosa cells and thus also correcting hyperandrogenism in a rat model of PCOS [64]. EA upregulates the numbers of pre-ovulatory follicles and corpora lutea by increasing innervation of blood vessels near the hilum [65]. The protective effect of EA may be through an upregulation of the IRS-1/PI3K/GLUT4 signaling pathway responsible for improving insulin resistance scores

 https://doi.org/10.3831/KPI.2023.26.2.105

[61]; and by inactivating the mTOR/4E-BP1 signaling pathway in a rat model of PCOS [66]. EA improves insulin sensitivity in PCOS models, associated with enhanced plasma insulin-like growth factor-I concentrations, amplified expression of leptin and interleukin-6 (IL-6) and diminished expression of uncoupling protein 2 in visceral adipose tissue [67], inducing the activation of the AMPK pathway to suppress SREBP-1 expression, and finally inhibiting insulin resistance, mitochondrial dysfunction and oxidative stress in a PCOS rat model [68]. EA also ameliorated insulin sensitivity in soleus muscle and mesenteric adipose tissue, whereas manual acupuncture acted more efficiently upon glucose tolerance [69]. In summary, acupuncture therapy has a positive impact on the treatment of PCOS through the following mechanisms: sympathetic output modulation through spinal reflexes [70], modulation of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes through the increase of β-endorphin secretion [71-74], and influencing insulin sensitivity or resistance through functional balance of the insulin signalling pathways [69].

The aim of this work was to review recent publications of acupuncture treatment reported in patients with PCOS, based on the literature produced in the English language from 2015 to 2020. We also included information from the main databases for randomized (RCT) and non-randomized controlled trials (non-RCT) and attested to acupuncture efficacy in resolving the main symptoms of PCOS regarding endocrine, metabolic, and emotional features.

METHODOLOGY

1. Search strategy for identification of studies

The objective of this work was to review and analyze the literature on the efficacy of acupuncture treatments applied to women with PCOS from September 2015 to September 2020, written in English. For assessing and reviewing the published scientific papers, an online search in EBSCO, Cochrane, PubMed, Medline, Embase and CINHAL databases was performed following the PRISMA protocol and its flowchart. Keywords used followed a conjugated series of semantics that allow optimized cross-referencing:

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) …

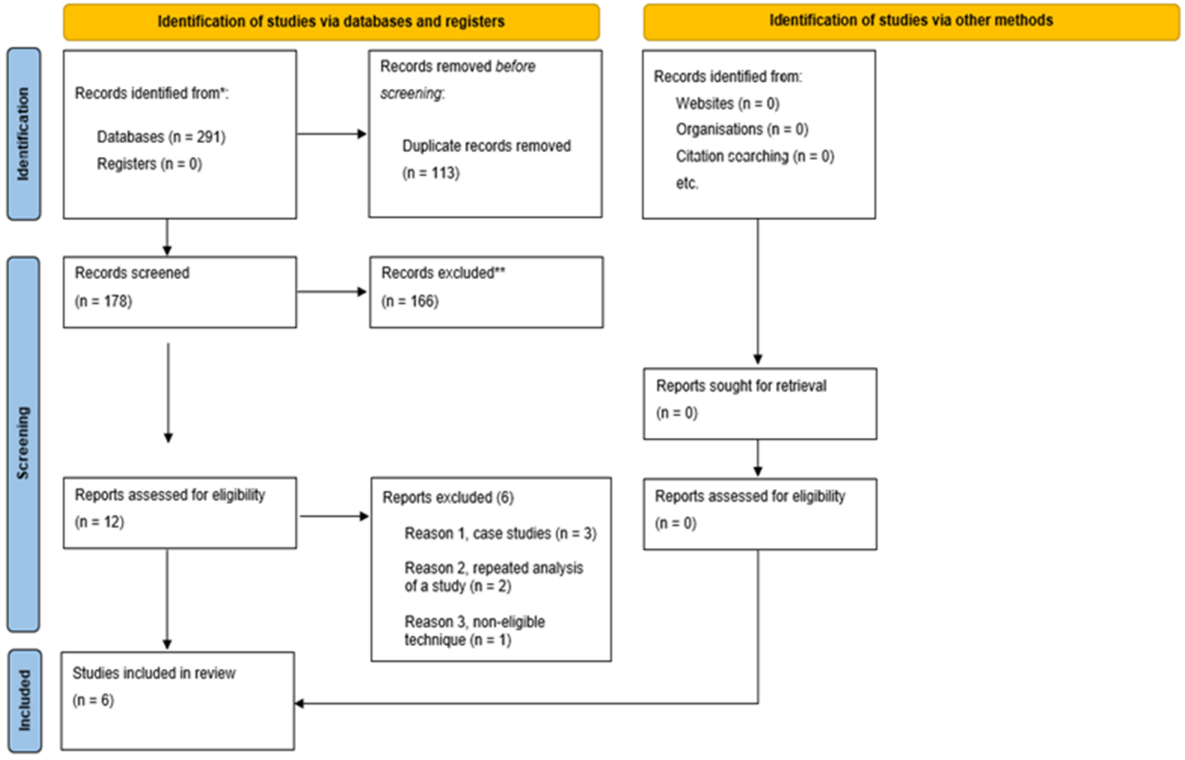

Figure 1. Description of the search protocol followed in this research.

(a) Acupuncture OR acupuncture therapy OR acupuncture treatment

(b) TCM OR Traditional Chinese Medicine

2. Inclusion/exclusion criteria

The Population, Intervention, Comparison, Outcome and Study (PICOS) strategy was employed [75].

1) Study participants

Women diagnosed with PCOS as assessed through the Rotterdam guidelines or with a PCOS diagnosis following the AE-PCOS criteria were included. The patient screening had no age or ethnicity restrictions.

2) Study intervention

Acupuncture was used as an eligible intervention, primarily as acupuncture techniques with needle penetration at acupoints, such as body acupuncture or electroacupuncture, and non-invasive techniques, such as laser acupuncture.

3) Study comparison

The control interventions included conventional Western medicine treatment like hormonal birth control or clomiphene prescription, lifestyle interventions and/or acupuncture at unrelated acupoints/sham acupuncture.

4) Study outcome measures

Primary outcomes included three aspects: metabolic parameters (body mass index [BMI], waist-hip ratio [WHR], Homeo-

Table 1. Eligible articles for review according to the inclusion/exclusion criteria

No.	RCT study	Type of treatment	Objective	Duration	Outcome
1	El-Mekawy et al., 2015 [77]	Laser acupuncture + healthy lifestyle	Evaluate the effect of laser acupuncture combined with a diet-exercise intervention on obese polycystic ovarian females	3 months	Laser acupuncture combined with the diet-exercise intervention improves both metabolic and endocrine features as well as increasing the follicular size in obese polycystic ovarian women
2	Hong et al., 2016 [78]	Manual acupuncture alone or with clomiphene	Observe clinical efficacy of acupuncture on the treatment of anxiety disorder in PCOS women and compare with Clomiphene therapy alone and combined with manual acupuncture	3 months	Combination of manual acupuncture with clomiphene treatment showed better results regarding anxiety treatment when compared with clomiphene or acupuncture alone
3	Wu et al., 2017 [79]	Manual and electroacupuncture alone or with WM therapy – clomiphene	Test the effect of acupuncture and/or clomiphene in infertile PCOS women	4 months	Acupuncture with or without clomiphene didn't increase live births
4	El-Shamy et al., 2018 [80]	Laser acupuncture + healthy lifestyle	Test the effect of laser acupuncture on the improvement of PCOS symptoms	3 months	Results suggest Laser acupuncture as a safe alternative or complement to WM in ovulatory induction
5	Cao et al., 2019 [81]	Manual and electroacupuncture (Tung protocol)	Assess the efficacy of Tung acupuncture as a complementary treatment to PCOS women to regulate sex hormones disruptive secretion	3 months	Tung acupuncture showed no improvement on LH/FSH ratio but might have some long-term effect over weight management and menstrual frequency on PCOS women
6	Wang et al., 2019 [82]	Manual and electroacupuncture	Evaluate the efficacy of electroacupuncture (EA) for the treatment of anxiety and depression in unmarried patients with polycystic ovarian syndrome (PCOS) by secondary analysis of a randomised controlled trial	4 months	EA appears to improve symptoms of anxiety/depression and quality of life in PCOS patients and may influence serum levels of NE and 5-HT

Resume of the results by full text search regarding acupuncture treatment.

 https://doi.org/10.3831/KPI.2023.26.2.105

Table 2. Description of study design/protocols for the intervention and control groups

No.	Population	Study design	Study group protocol	Control group protocol
1 [77]	28 obese post-menopausal women were randomly distributed	Laser acupuncture + healthy lifestyle	• Diet-exercise intervention (*vide* next column) • Sessions of laser acupuncture, 3 times/week for 12 weeks. It was applied with an energy density of 2 J/cm^2, a pulse radiation of 200 ns, and with a pulse frequency of 5,000 Hz. The laser was applied to the skin vertically with irradiation time 2 min/point, 3 times/week for 12 weeks. CV4 (Guanyuan); CV9 (Shuifen); CV12 (Zhongwan); ST25 (Tianshu); ST36 (Zusanli); ST40 (Fenglung); SP6 (Sanyinjiao)	• Diet-exercise intervention An energy-restricted diet for 12 weeks. First, the recommended daily kilocalorie intake was computed by multiplying the Harris-Benedict equation by 1.55. Then, daily energy intake was restricted by 1,000 kcal/day (daily kilocalorie requirement-1,000 kcal). The diet caloric proportion of the protein, fat and carbohydrate was set at 15%, 30% and 55% respectively.
2 [78]	90 PCOS women, aged of 20-40 years old intended for infertility, were randomly allocated into groups of 30 each: Group A: acupuncture combined with clomiphene group, Group B: acupuncture group Group C: clomiphene group	Manual acupuncture alone or with clomiphene	• Group B - Treatment with acupuncture only: Fast rotating needling insertion method and mild reinforcing-reducing manipulation were operated. The needle retaining time was 30 minutes. Protocol of acupoints: BL18 (Ganshu), BL23 (Shenshu), CV3 (Zhongji), CV4 (Guanyuan), CV9 (Shuifen), CV12 (Zhongwan). Back-Shu points were used alternatively. • Group A - Treatment with acupuncture and clomiphene Acupuncture protocol (vide upper line) Clomiphene protocol (vide next column)	• Group C- Treatment with clomiphene only: Protocol - Patients received 50 mg CC 1 time/day from the 1st day to 5th day of the menstrual cycle in the 1st month. Without ovulation, the dose of CC was increased to 100 mg during the 2nd cycle and 150 mg in the 3rd cycle. Otherwise, the CC should be maintained the same dose with the previous month. The patients wouldn't take CC in the followed-up month. The drug treatment should be ceased once pregnancy was confirmed.
3 [79]	1,000 PCOS diagnosed women randomly distributed within 4 groups of 250 patients each: Group A: active acupuncture + clomiphene Group B: sham acupuncture + clomiphene Group C: active acupuncture + placebo Group D: sham acupuncture + placebo	Manual and electroacupuncture alone or with WM therapy - clomiphene	• Group A – Manual and electroacupuncture with CC Acupuncture protocol - in the active acupuncture protocol, 2 sets of acupuncture points were alternated every other treatment (vide line below) Clomiphene protocol - patients started with an initial oral dose of 50 mg of clomiphene/placebo from days 3 to 7 of the menstrual cycle. The dosage of oral medication was increased by 50 mg in the absence of ovulation or maintained in the presence of ovulation. The maximum dosage of clomiphene/placebo did not exceed 150 mg/day or 750 mg/cycle. The treatment could be repeated for up to 4 cycles.	• Group B – Sham acupuncture with CC Sham acupuncture protocol: 2 needles were inserted superficially to a depth of less than 5 mm, 1 in each shoulder and 1 in each upper arm at non-acupuncture points, and needles were not stimulated manually when inserted. Thereafter, the 4 needles were attached to electrodes and the stimulator was turned on to mimic the active acupuncture but with no electrical stimulation. Clomiphene protocol – vide previous column

Table 2. Continued

No.	Population	Study design	Study group protocol	Control group protocol
			• Group C – Manual and electroacupuncture with placebo Active acupuncture protocol Protocol 1: CV3 (Zhongji), CV6 (Qihai), and ST29 (Guilai) bilaterally SP6 (Sanyinjiao), and SP9 (yinlingquan) bilaterally – thereafter connected to and electrical stimulator and stimulated with low frequency EA of 2 Hz, 0.3 ms pulse length. Needles also placed in LI4 (Hegu) bilaterally and GV20 (Baihui) were manually stimulated to evoke needle sensation every 10 min, in total 4 times. Protocol 2: ST25 (Tianshu) and ST29 (Guilai) bilaterally, SP6 (Sanyinjiao) and LR3 (Taichbng) bilaterally with electrical stimulation. CV3 (Zhongji), CV6 (Gihai), PC6 (Neiguan), GV20 (Baihui) by manual stimulation as described above. Placebo Protocol – vide upper line	• Group D – sham acupuncture with placebo Acupuncture protocol – vide line above Placebo protocol – vide upper line, previous column
4 [80]	25 PCOS women were randomly allocated to a laser acupuncture or the control group	Laser acupuncture + healthy lifestyle	• Laser acupuncture treatment: two sessions per week during first 2 weeks, one session/week through 4 weeks, and one session every second week for 6 weeks, in a total of 11 sessions through 12 weeks. An infrared laser with 830 nm wavelength, 10 mW power output and 0.5 J energy was applied 60 seconds for each acupoint. The head of the machine was used perpendicularly, with direct contact to each point. CV4 (Guanyuan), Unilateral; CV5 (Shimen), Unilateral; ST29 (Guilai, Bilateral), SP6 (Sanyinjiao, Bilateral) • Healthy lifestyle: vide next column	• Sham laser acupuncture: women in the control group were treated by placebo laser acupoints • Healthy lifestyle Advice for a low carbohydrate and high-protein diet, high dietary fibre, and less saturated fat, were instructed to engage in aerobic exercise, fast walking for at least 30-45 min, 3 days a week.

static Model Assessment of Insulin Resistance [HOMA-IR] and lutenizing hormone/follicle stimulating hormone [LH/FSH], triglycerides [TG] and low-density lipoprotein [LDL]), endocrine and reproductive (secretion of sex hormones, menstrual frequency, live births, ovulation induction and follicular size) and mental health variables (anxiety/depression, scales of quality of life).

5) Study design

Randomized clinical trials and non-RCT of acupuncture treatments were included. Non-TCM complementary medicine, non-English language, unavailable full text, media communications, protocol and case studies, meta-analysis and reviews were disregarded. *In vivo* and *in vitro* preclinical studies were ruled out as PCOS in humans has high emotional and psychiatric components with social implications [76]. Despite the value of their biochemical contribution, these *in vivo* and *in vitro* studies lack emotional and psychiatric components.

Table 2. Continued

No.	Population	Study design	Study group protocol	Control group protocol
5 [81]	60 PCOS diagnosed women, aged 18-45 years old, were randomly assigned to an acupuncture group (n = 30) or cyproterone acetate/ethinylestradiol (CPA/EE) group (n = 30).	Manual and electroacupuncture (Tung protocol)	• Acupuncture protocol The study used Tung's acupoints Fuke, Huanchao, Tianhuang, Renhuang, as well as the traditional acupoints CV4 (Guanyuan) and EX-CA1 (Zigong). The locations of these acupuncture points matched the recommendations of Tung's acupuncture. Fuke point iis on the ulnar side of the proximal joint of thumb. There are two points up and down, 1/3 of the distance away from the interphalangeal joint. Huanchao point is on the middle of the central joint, on the ulnar side of ring finger. Tianhuang point is at the same location of SP9 (Yin Lingquan). Renhuang point is at the same location of SP6 (San Yinjiao). EX-CA1 is at the same location as CV19 (Zigong). Bilateral EX-CA1 points were connected to an electrical stimulator with a continuous wave of 20 Hz, and the current intensity was slowly increased to a slight tremor in the abdomen. At the same time, a moxa box was administered via smokeless moxa stick on the lower abdomen for 30 min.	• Contraceptive pill group CPA/EE (Diane-35, Bayer Healthcare Co. Ltd) was taken orally one tablet per day from the 8th day of menstrual cycle or any day for patients with amenorrhea. The pills were administered for 21 days consecutively, stopped for seven days, and on the eighth day, continued to take the pills again for 3 menstrual cycles (28-day cycles).
6 [82]	54 PCOS diagnosed women, aged 18-28, were randomly assigned to an acupuncture treatment group or control group.	Manual and electroacupuncture	• Acupuncture protocol Two sets of acupuncture points were alternately needled, with twice sessions/week for 16 weeks. The first set of acupuncture points comprised CV3 (Zhongji), CV6 (Qihai), bilateral ST29 (Guilai), bilateral SP6 (Sanyinjiao), bilateral SP9 (Yinlingquan), bilateral LI4 (Hegu) and GV20 (Baihui). The second set of acupuncture points consisted of CV3 (Zhongji), CV6 (Qihai), bilateral ST25 (Tianshu), ST29 (Guilai), SP6 (Sanyinjiao), bilateral LR3 (Taichong), bilateral PC6 (Neiguan) and GV20 (Baihui). Needles inserted at GV20 (Baihui), PC6 (Neiguan) and LI4 (Hegu) were stimulated manually to evoke needle sensation (de qi) including soreness, numbness, or distension around the puncture sites. The other acupuncture points were connected to an electrical stimulator. Stimulated at low (2 Hz) frequency (0.3 ms pulse width).	• Sham acupuncture protocol There were 4 needling points (2 on the shoulders and 2 on the upper arm) at locations anatomically remote from the verum acupuncture needling sites. Electrodes were attached to the needles and the stimulators were turned on, to mimic current in the acupuncture protocol, but set at an intensity of zero.

Brief description of the protocol's design for the intervention and control groups of each study.

6) Database extraction

Data were extracted from the database sites to the Endnote application where the screening to titles and abstracts was performed after eliminating the duplicates. Database search was completed by the main researcher of the project.

MAIN RESULTS AND DISCUSSION

Six studies were considered for final analysis [77-82] from the 178 studies initially selected (Fig. 1). These six studies cast an initial total of 1,257 patients and comprised objectives within symptom management in women with PCOS. Table 1 synthesizes the main information of each study: publication year, main author, primary objective, duration of the study, type of treatment and principal outcomes.

One of the studies (16.7%) focused on the rebalance of altered secretion of sex hormones [81] whereas another (16.7%) focused on weight management and the effect on the metabolic

Table 3. Considered outcomes per eligible study

Study	Outcomes evaluation criteria
1 [77]	BMI + WHR + Follicular size + FBI + LH + FSH + LH/FSH + AMH
2 [78]	SAS (self-rating anxiety scale) + ovulation rate
3 [79]	Live births + BMI + WHR + Bloodwork (FSH + LH + LH/FSH + TT + Oestradiol + Progesterone + TG + TC + HDL + LDL)
4 [80]	Bloodwork (FT + TT + FSH + LH + LH/FSH + AMH + HOMA-R)
5 [81]	LH/FSH (primary outcome) + LH + FSH + TT + BMI + ovary volume + ovary cysts number + frequency of menstruation
6 [82]	Serum levels of neurotransmitters (AD/NE/5-HT/GABA) + scores of SAS, SDS, SF-36, PCOSQOL, CHQOL

Outcome criteria for each study.

AMH, Anti-Müllerian hormone; BMI, body mass index; WHR, waist hip ratio; FBI, fasting blood insulin; HDL, high density lipoprotein; HOMA-IR, Homeostatic model assessment for insulin resistance; LH, luteinizing hormone; FSH, follicular stimulating hormone; TC, total cholesterol; TG, triglycerides; TT, total testosterone; AD, epinephrine; 5-HT, serotonin; GABA, γ-aminobutyric acid; NE, norepinephrine; SAS, self-rating anxiety scale; SDS, Zung's self-rating depression scale; SF-36, Medical outcomes study questionnaire; PCOSQOL, The PCOS quality of life scale; CHQOL, Chinese quality of life questionnaire.

Table 4. Comparison of outcomes in studies measuring endocrine and reproductive features

					Endocrine features				
Study		Sample size	PCOS diagnostic	Intervention	Age (avg)	LH (mUI/mL)	FSH (mUI/mL)	LH/FSH	TT (ng/dL)
3 [79][a]	*B*	250	Rotterdam	Electroacupuncture + Manual acupuncture + CC (32 sessions)	28 (27 CG)	10.6 (10.0 CG)	6.1 (6.2 CG)	1.8 (1.6 CG)	48.4 (47.8 CG)
	A	235				8.3 (7.2 CG)	3.5 (3.7 CG)	2.4 (1,9 CG)	66.3 (68.6 CG)
3 [79][b]	*B*	250	Rotterdam	Electroacupuncture + Manual acupuncture (32 sessions)	28 (28 CG)	10.6 (10.8 CG)	6.1 (6.0 CG)	1.8 (1.9 CG)	48.3 (47.3 CG)
	A	223				10.3 (10.5 CG)	4.8 (4.8 CG)	2.1 (2.2 CG)	52.0 (52.2 CG)
4 [80]	*B*	13	AE-PCOS	Laser acupuncture + Lifestyle changes (11 sessions)	20 (19 CG)	10.1 (11.3 CG)	4.9 (5.0 CG)	2.4 (2.5 CG)	69.5 (66.3 CG)
	A	11				6.9 (9.9 CG)	4.8 (4.8 CG)	1.3 (2.0 CG)	60.0 (63.0 CG)
5 [81]	*B*	30	Rotterdam	Electroacupuncture + Manual acupuncture (24 sessions)	29 (28 CG)	10.7 (13.2 CG)	5.1 (5.6 CG)	2.1 (2.4 CG)	63.4 (53.6 CG)
	A	27				8.7 (10.8 CG)	5.1 (5.0 CG)	1.7 (1.9 CG)	50.5 (48.7 CG)

Endocrine outcomes of articles 3, 4 and 5.

Avg, average; CC, clomiphene citrate; LH, Luteinizing hormone; FSH, Follicle stimulating hormone; LH/FSH, ratio of Luteinizing Hormone over Follicle Stimulating Hormone; TT, Total Testosterone.

B – Before treatment. A – after treatment.

CG – Control group values. CG1 – Treatment with catgut embedding only. CG2 – Treatment with decoction only.

[a]Treatment with active acupuncture + clomiphene, CG – Control acupuncture + clomiphene.

[b]Treatment with active acupuncture + placebo, CG – Control acupuncture + placebo.

 https://doi.org/10.3831/KPI.2023.26.2.105

changes affecting obese women diagnosed with this condition [77]. Another two studies (33.3%) focused on the reproductive issues that affect women with PCOS of fertile age [79, 80] and the remaining two (33.3%) approached PCOS-induced anxiety [78, 82]. Four of the six studies (66.7%) had a period of acupuncture treatment of three months [77, 78, 80, 81] and the other 33.3% had a treatment period of four months [79, 82], regardless of the follow-up period. Four studies (66.7%) tested the effect of acupuncture treatment in combination with other interventions: Western medicine (WM) therapeutics [78, 79] or simply lifestyle alterations [77, 80]. Among the different acupuncture techniques, laser acupuncture was used in 33.3% of the studies [77, 80], manual acupuncture and electro-acupuncture in 50.0% [79, 81, 82] and single manual acupuncture in 16.7% [78] of studies. The main and secondary outcomes of each study varied according with the main objective. Table 2 describes the study design of each eligible work and Table 3 sums up the main outcome criteria for each study.

For a better comparison of the results among the studies, information was gathered within the scope of the main group of outcomes of each study: endocrine and reproductive features (Table 4), metabolic features (Table 5) and mental health features (Table 6). In these tables we only mentioned the final values obtained for both intervention and control groups. The baseline values were referred to in the *Discussion* section, if appropriate.

1. Endocrine and reproductive features

Endocrine implications of PCOS syndrome include deviations to the guideline values of serum steroid hormones, which is usually clinically associated with hyperandrogenism and/or reproductive health problems. Acupuncture alone or in combination with other treatment approaches was used to improve

Table 5. Comparison of outcomes in studies measuring metabolic features

					Metabolic features						
Study		Sample size	PCOS diagnostic	Intervention	Age (avg)	BMI	WHR	HOMA-IR	LH/FSH	TG (mmol/L)	LDL (mmol/L)
1 [77]	*B*	30	Rotterdam	Laser acupuncture + healthy lifestyle (36 sessions)	54 (53 CG)	39.9 (42.8 CG)	0.95 (0.95 CG)	13.3† (10.3 CG)	-	1.8 (1.8 CG)	3.5 (3.2 CG)
	A	30				35.5 (38.7 CG)	0.94 (0.95 CG)	9.3† (8.6 CG)		1.4 (1.4 CG)	3.4 (3.2 CG)
3 [79][a]	*B*	250	Rotterdam	Electroacupuncture + manual acupuncture + CC (32 sessions)	28 (27 CG)	23.8 (24.4 CG)	0.90 (0.90 CG)	3.2 (3.0 CG)	1.8 (1.6 CG)	1.5 (1.6 CG)	2.9 (3.0 CG)
	A	235				23.8 (24.3 CG)	0.90 (0.90 CG)	3.4 (3.7 CG)	2.4 (1.9 CG)	1.3 (1.6 CG)	2.7 (2.7 CG)
3 [79][b]	*B*	250	Rotterdam	Electroacupuncture + manual acupuncture (32 sessions)	28 (28 CG)	24.2 (24.6 GC)	0.90 (0.90 CG)	3.3 (3.0 CG)	1.8 (1.9 CG)	1.7 (1.5 CG)	3.1 (2.9 CG)
	A	223				24.0 (24.4 CG)	0.90 (0.90 CG)	3.6 (2.9 CG)	2.1 (2.2 CG)	1.5 (1.5 CG)	3.0 (2.8 CG)
4 [80]	*B*	13	AE-PCOS	Laser acupuncture + lifestyle changes (11 sessions)	20 (19 CG)	27.2 (26.9 CG)	-	2.9 (2.5 CG)	2.4 (2.5 CG)	-	-
	A	11				25.6 (25.0 CG)		1.9 (1.5 CG)	1.3 (2.0 CG)		

Comparison of the main outcomes between study groups of articles 1,3, and 4 (mostly focused on metabolic features).
LH/FSH, ratio of Luteinizing Hormone over Follicle Stimulating Hormone; BMI, Body mass index; WHR, waist hip ratio; HOMA-IR, Homeostatic model assessment for insulin resistance; TG, triglycerides; LDL, Low density lipoprotein.
B – Before treatment. A – after treatment.
CG – Control group values.
[a]Treatment with active acupuncture + clomiphene, CG – Control acupuncture + clomiphene.
[b]Treatment with active acupuncture) + placebo), CG – Control acupuncture + placebo.
HOMA-IR values for study 3 were calculated directly from the HOMA-IR algorithm online - article data were not coherent.
†Values of serum insulin mIU/L (no HOMA-IR or Fasting glucose data available).

Table 6. Comparison of outcomes in studies measuring mental health features

Mental health features											
Study		Sample size	PCOS diagnostic	Intervention	Age	BMI	SF36	PCOSQOL	SDS	SAS	Ovulation rate (%)
2 [78]	*B*	30	Rotterdam	Manual acupuncture + CC (36 sessions)	(20-40 range)	-	-	-	-	59.55 (57.36 CG1 56.57 CG2)	86.21
	A	29								34.93 (37.46 CG1 52.29 CG2)	
3 [79][a)]	*B*	250	Rotterdam	EA + manual acupuncture + CC (32 sessions)	28 (27 CG)	23.8	78.0 (77.6 CG)	4.4 (4.4 CG)	43.7 (44.4 CG)	42.0 (42.5 CG)	90.0 (93.4 CG)
	A	235				23.8	76.5 (74.9 CG)	4.4 (4.4 CG)	45.3 (45.1 CG)	42.3 (43.2 CG)	
3 [79][b)]	*B*	250	Rotterdam	EA + manual acupuncture (32 sessions)	28 (28 CG)	24.2	77.0 (78.2 CG)	4.4 (4.4 CG)	44.7 (44.6 CG)	42.6 (42.4 CG)	70.0 (69.8 CG)
	A	223				24.0	74.7 (76.9 CG)	4.4 (4.4 CG)	45.8 (45.3 CG)	44.9 (42.4 CG)	
6 [82]	*B*	23	Rotterdam	EA + manual acupuncture (32 sessions)	(18-28 range)	-	60[‡] (64 CG)	3.2[§] (3.2 CG)	37 (38 CG)	38 (42 CG)	-
	A	23					68[‡] (68 CG)	3.8[§] (3.5 CG)	33 (42 CG)	35 (43 CG)	

Comparison of the main outcomes between study groups of articles 2, 3 and 6 (mostly centred on mental health features).
EA, Electroacupuncture.
B – Before treatment. A – after treatment.
CG – Control group values. CG1 – treatment with acupuncture alone. CG2 – treatment with clomiphene alone.
[a)]Treatment with active acupuncture + clomiphene, CG – Control acupuncture + clomiphene
[b)]Treatment with active acupuncture + placebo), CG – Control acupuncture + placebo
Study 7 – CG: sham acupuncture
[‡]Mental Health score alone from the whole SF36 (Short Form Health Survey).
[§]Emotion score alone from the whole PCOSQOL (PCOS quality of life questionnaire).
SDS – Social Depression.
SAS – Social Anxiety.

symptoms and restore hormonal function (Table 2, 4).

Studies 3, 4 and 5 measured outcomes such as LH, FSH and testosterone (free and total serum testosterone) in women with PCOS, usually correlated with ovarian dysfunction and subfertility/infertility [83]. The diagnosis of polycystic ovary syndrome was assessed through the Rotterdam guidelines in studies 3 and 5, and the participants had an average age of 28 to 29 years. In study 4, women were an average of 20 years old and the PCOS diagnosis followed the AE-PCOS criteria, which ignores the Rotterdam phenotype D/4 (non-hyperandrogenic PCOS).

Laser intervention related to a healthy lifestyle (study 4), manual and electroacupuncture alone (group C of study 3, and study 5) and linked to WM intervention such as clomiphene intake (group A of study 3) were the interventions explored to recognize the impact on the metabolic and endocrine features of the syndrome in these studies.

Both blood values of total testosterone (TT) and LH improved significantly in studies 4 and 5 – LH values also recovered in study 3, but TT values worsened. Comparing studies 4 and 5, the TT values were enhanced in the electroacupuncture plus manual acupuncture intervention (study 5) as compared to the laser intervention (study 4). Both electroacupuncture (study 5) and laser treatment plus lifestyle intervention (study 4) groups showed a better advancement in the TT values when compared with both CGs, contraceptive pill intake alone or lifestyle changes alone. In study 5, in comparison with study 4, it was possible to identify: 1) a higher number of treatment ses-

 https://doi.org/10.3831/KPI.2023.26.2.105

sions; 2) a regular gap between sessions throughout the treatment period; and 3) a lesser number of acupoints related to ovarian innervation within the study protocol.

On the other hand, a higher impact of the decrease in LH peak values was observed in the laser plus lifestyle intervention group (study 4) followed by the CG treated with contraceptive pill (CG of study 5), electroacupuncture and manual acupuncture intervention (study 5) and finally lifestyle intervention alone (CG of study 4). This tendency was opposed to what was determined from the analysis of the reduction in FSH values, which was significant in the CG of study 3, followed by the CG of study 4, women treated with laser acupuncture and lifestyle intervention (study 4) and, finally, by electroacupuncture and manual acupuncture intervention (study 5). With less sessions of laser intervention, the protocol in study 4 included four points of the conception vessel related to ovary innervation (CV4 and CV5, SP6 and SP9) as compared to three acupoints in study 5 (CV4, SP6 and SP9). For example, this could indicate a possible positive relationship to the laser activation of the acupoints in the regulation of the sympathetic nervous system by GnRH dependent release of LH, through the downregulation of the HPG axis.

Overall, the results attained with the different types of acupuncture follow a positive regulation of the hormonal status of women diagnosed with PCOS – showing a higher impact within TT regulation and LH regulation. Because the FSH values did not exhibit a significant change when compared to the LH secretion in the studies (Table 5), we may infer that the LH/FSH adjustment within the acupuncture interventions is mostly due to LH status regulation.

Taking into account the current description of the PCOS diagnosis with respect to the Rotterdam criteria, Hsu identified four possible phenotypes according to symptom severity [84]. This might impact the results as a treatment maybe more oriented towards specific TCM syndromes associated with a specific to Rotterdam phenotype. Therefore, the evaluation of these results should proceed with caution, always taking into consideration if the studies are in conformity or not with the Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) [85], and/or the Consolidated Standards of Reporting Trials (CONSORT) [86] quality criteria for Traditional Chinese Medicine. Regarding the quality of the studies analyzed, only study 3 most closely pursued STRICTA recommendations. Studies 3, 4 and 5 were RCTs, but only study 3 was multicentered and followed the 2 × 2 factorial design. Most of the RCTs are two-arm designs with a single intervention arm as compared to a single control group (as in study 4) or searching for new treatment combined strategies [87].

2. Metabolic features

Studies 1, 3 and 4 had bloodwork outcomes regarding individual metabolic status before and after the respective treatments (Table 5). Study 1 regarded post-menopausal women whereas studies 3 and 4 focused on women at reproductive age. With respect to sample size, the sample of study 4 was much larger than the others.

To facilitate interstudy comparison, criteria were selected that covered most of the outcomes: BMI, WHR, HOMA-IR and the LH/FSH, TG and LDL (Table 3). Baseline BMI was similar among the studies, except for study 1, which was was higher, and can be explained by the fact that the sample consisted of older women and, according to the literature, metabolic issues derived from the PCOS condition are detected more frequently at older age [19, 25, 84, 88-90]. It is possible to observe a BMI reduction gap in studies 1 and 4 in comparison to study 3. Comparing studies 1 and 4, an improvement of BMI values was observed in study 1, even with both trials using laser acupuncture plus lifestyle changes as intervention. Differences between studies 1 and 4 that could justify these results could be: 1) in study 1, women were assisted and followed by the research team throughout the entire exercise and diet plans, rather than only briefing and recommending to patients the importance of a healthy hypocaloric diet in combination with daily exercise used in study 4; 2) study 1 had a total of 36 interventive sessions compared to 11 in study 4; 3) the study 1 protocol of acupuncture had a higher number of acupoints related to the regulation of metabolic function, such as ST25, ST36, ST40 and SP6 as compared to the choice of only two points – ST29 and SP6 – in study 4; and 4) the occurrence of laser radiation at each acupoint was 120 seconds in study 1 as compared to 60 seconds in study 4. In addition, study 4 did not present the values of WHR which would have been a valuable assessment for comparison.

Study 1 used serum insulin as a biomarker for spleen metabolic function instead of HOMA-IR, which was utilized in studies 3 and 4. Serum insulin displayed remarkable improvement in the laser acupuncture associated with lifestyle intervention in comparison with the lifestyle changes alone. On the other hand, the values of HOMA-IR demonstrated a similar enhancements comparing the laser acupuncture with lifestyle changes and life-

style change only (study 4 groups). The difference in the protocols of study 1 and 4 interventions, already mentioned above, could justify the improved values in the groups of study 1.

The metabolic syndrome developed in females diagnosed with PCOS has been linked to endocrine changes, namely with LH/FSH values. For that reason, these values are presented in Table 5, where it is possible to verify that in study 4, the improvement of HOMA-IR values in both intervention and CG, were accompanied by a betterment of LH/FSH values also in both groups (underscoring a greater advancement for the intervention group). LH/FSH was not measured as a biomarker in study 1 as it involved a population of climacteric women. Study 3 considered both HOMA-IR and LH/FSH values. The results reported in the studies may confirm the relationship between glucose metabolism and the endocrine functional status of the HPG axis, namely in the index of LH/FSH.

It would be interesting to compare the changes in fat metabolism using the same kind of acupuncture intervention. However, study 4 did not measure TG and LDL. It is only possible to compare these markers between studies 1 and 3. Therefore, study 1 illustrated that the impact of lifestyle changes alone (CG) had no impact on the LDL values, and there was remarkable reduction in the TG values. This decline in TG values was the same for the control and the laser acupuncture intervention groups, and the intervention group indicated an improved reduction in the LDL values when compared to the control group – this appears to show a marked impact of laser treatment for cholesterol regulation in comparison to triglyceride metabolism. However, this progression in LDL in comparison to TG metabolism by acupuncture intervention was not confirmed in study 3, as it was used as electroacupuncture plus manual acupuncture instead of laser intervention. Table 5 reveals that CC intake alone or sham acupuncture had no difference in TG values, whereas the intervention with acupuncture alone or in combination with CC intake demonstrated an improvement in TG levels.

From the results summarized in Table 5 regarding TG and LDL metabolism, it is possible to conclude that active acupuncture alone improved TG values and CC association did not improve it. In addition, CC intake significantly enhanced LDL values as compared to the no active and sham acupuncture groups. The differences in outcomes between studies 1 and 3 can be derived from an aged population with different endocrine profiles in study 1 and differences in treatment protocols. Study 3 used LI4 and LR3, which could have facilitated the metabolism of TG even without the positive input of the lifestyle interventions of the study 1 population. Despite the distinct types of acupuncture used in these studies, an overall positive physiological response in biomarkers for women with PCOS when compared with baseline values and control groups was observed, except for WHR, HOMA-IR and LH/FSH in study 3. In the absence of HOMA-IR, TG and LDL values in study 1, the values of fasting serum insulin indicated a significant decrease after laser acupuncture combined with physical exercise and a restricted calorie diet.

In our opinion, future acupuncture studies should use secondary outcomes to describe the metabolic status of each phenotype before and after the respective treatment. This way, it would be possible to demonstrate an accurate assessment of acupuncture as a valuable tool for improving one of the most important features of women with PCOS. Both metabolic and infertility issues bring a financial burden to healthcare systems; however, metabolic syndrome brings more disruption of quality of life in the long run, whereas infertility issues are restricted to the fertile age range. Last, but not least, to facilitate interstudy comparability, it is important to create a standard for metabolic status criteria and to report respective international measures. We believe that researchers should follow STRICTA recommendations to increase study quality as more trials regarding POCS are being registered at the International Clinical Trials Registry Platform (ICTRP) database.

3. Mental health features

From the six studies selected for this review, studies 2, 3 and 6 measured mental health outcomes (Table 6). Study 3 counted them as secondary outcomes and as exploratory results of a study primarily focused on the effect of acupuncture as a complementary technique to clomiphene administration in women with PCOS having fertility issues. Studies 2 and 6 attended to the effect of acupuncture as a method to relieve the anxiety of women with PCOS with fertility issues and to manage anxiety and depression symptoms in unmarried women with PCOS, respectively.

Studies 2 and 6 used SAS as one of the outcome measures, although study 6 also assessed quality of life scores through PCOSQOL and SF-36. Additionally, study 6 rated depression in the patients through SDS (Table 3). In study 6, the control group was treated with sham acupuncture, whereas in study 2, three groups were utilized, a study group taking clomiphene +

acupuncture treatment, a CG with only active acupuncture, and another control group only taking clomiphene.

Overall, study 2 revealed that acupuncture associated with clomiphene improved anxiety scoring as compared to the use of acupuncture or CC alone (Table 6). The CG of this study (acupuncture treatment only) also exhibited a significant anxiety score reduction. Therefore, a CG with sham acupuncture and placebo would be important (as demonstrated in study 3, where the results indicated no anxiety improvement with active or sham acupuncture). The SAS questionnaire assessment in study 6 presented not only an improvement of anxiety values in the group under electroacupuncture intervention, but also a worsening of values in the control group submitted to sham acupuncture. A worsening of anxiety through SAS assessment was also noted in the groups of study 3 with higher indications of anxiety in the group treated with CC intake only, followed by the group treated with acupuncture only, the group treated with acupuncture combined with CC, and finally, the group treated with sham acupuncture and placebo. The difference in outcomes regarding anxiety among studies 2, 3 and 6 might be due to a longer treatment period in studies 3 and 6, possibly leading to a higher anxiety symptoms in the control groups when facing a lack of results and dealing with secondary effects of CC intake in study 3. In addition, study 2 used a differentiated protocol of acupoints where Shu points such as BL18 and BL23 were included in association to a group of four conception vessel acupoints (CV3, CV4, CV9 and CV12), in comparison to protocols that used only two conception vessel acupoints (CV3, CV6) correlated to stomach and spleen points and a couple of sedative acupoints (GV20, PC6, LR3). However, study 2 had a shorter period of intervention, but included a higher number of acupuncture interventions heightening the probability of better outcomes. Finally, the larger logistics implicated in study 3 could have influenced the bonding of women to the treatment, with possible feelings of pressure to oblige with the formalities.

PCOSQOL is the most recent questionnaire to assess the quality of life of women with PCOS [91] and it showed an amelioration of the emotion parameter with both interventions – active and sham acupuncture – of study 6. On the other hand, in study 3, PCOSQOL showed no changes in values for any group involved in the research. Mental health status measured through the SF-36 questionnaire confirmed an improvement in scoring after both active and sham interventions, with a higher impact on the group submitted to active acupuncture of study 6. Study 6 and study 3 each followed a close protocol of acupoints, techniques of acupuncture and a period of intervention. However, study 3 demonstrated a decrease in mental health status assessed through SF-36 which, like PCOSQOL, SAS and SDS parameters, could be attributed to several factors such as a) pressure to get pregnant, whereas in study 6, women did not have the intention of pregnancy or to directly address infertility issues; b) pressure of deadlines and several diagnosis requirements such as blood samples to be collected on due dates which was not required in study 6; and c) a larger scale of experiment, leading to a huge logistic management lacking time and resources to follow the patients closely, as compared to a smaller sample management in study 6. Considering the aforementioned information, it is possible to justify the confirmed correlation between the reduction of anxiety and depression scores in women submitted to acupuncture intervention in study 6 with a physiological decrease in serotonin and gamma-aminobutyric acid (GABA), and the increase of norepinephrine after active acupuncture treatment. Extra activation of the sympathetic nervous system by external pressures added to already existing internal pressure to pursue pregnancy, is a possible justification for a difference of anxiety and depression outcomes between studies 3 and 6.

Overall, the results of acupuncture treatment determined in studies 2, 3, and 6 were positive, with a significant improvement of anxiety management when combining acupuncture intervention with CC intake in women diagnosed with PCOS and aiming for pregnancy. From the gathered information, it seems to be a positive modulation of the nervous system with acupuncture techniques. Despite the favorable results of the treatments on PCOS mental health status, current scenarios demand more acupuncture studies with a broader range of outcomes in a standardized manner, that obtain accountable conclusions. With respect to the quality of the studies, only study 3 followed STRICTA recommendations.

CONCLUSION

According to the reviewed literature, with respect to endocrine outcomes addressing the treatment of ovarian dysfunction present in women with PCOS, we identified that a higher success for acupuncture was related to: 1) a higher number of treatment sessions; 2) a regular gap between sessions throughout the treatment period when balancing TT; 3) a preference for protocols with a higher number of acupoints related to ovarian innervation for balancing LH or LH/FSH, regardless of the

number and frequency of treatment sessions; 4) FSH modulation appears more affected by a healthy lifestyle and CC intake than by acupuncture treatment.

With respect to the outcomes of metabolic biomarkers addressed by acupuncture treatment, from the analyzed studies, we conclude that: 1) laser acupuncture with lifestyle intervention demonstrated greater improvement in biomarkers such as BMI, serum insulin and HOMA-IR; 2) the success of acupuncture treatment appears related to a higher number of sessions and longer stimulation with laser radiation of the acupoint; 3) for electroacupuncture treatments, the success is correlated with the number of spleen and stomach acupoints considered in the protocol; 4) laser acupuncture indicated a synergistic effect with a healthy lifestyle for serum insulin management; 5) the analyzed studies displayed a parallel variation between the values of HOMA-IR and LH/FSH; 6) FSH modulation is more influenced by CC intake or even the adoption of a healthy lifestyle, so the correlation between LH/FSH and HOMA-IR values might be linked to a direct influence of acupuncture on LH regulation as well as on insulin metabolism; 7) active electroacupuncture alone improved TG values, and CC intake association had no impact on them; and 8) CC intake significantly improved LDL values as compared to no active electroacupuncture and sham acupuncture groups.

Last but not least, when studying the effect of acupuncture intervention alone or in combination with other procedures on mental health issues related to PCOS, our review made it possible to infer that: 1) longer treatment period interventions seemed to worsen anxiety symptoms in the control groups; 2) a protocol of Shu points, such as BL18 and BL23 in combination with CV3, CV4, CV9 and CV12 was more effective addressing anxiety than protocols considering CV3 and CV6, plus stomach and spleen points and a few of sedative acupoints (GV20, PC6, and LR3); 3) a higher number of acupuncture sessions even performed for a shorter intervention period seemed to improve anxiety symptoms; 4) physiological decrease of serotonin and GABA, and an increase of norepinephrine after the active acupuncture treatment might be behind the positive effect on the psycho-emotional issues of women diagnosed with PCOS; and 5) a decrease of mental health status assessed through SF-36 could be attributed to several stress factors such as pressure for getting pregnant, external pressure of deadlines and diagnosis bureaucracy, lack of management time and resources to closely follow patients, which might lead to a sustained sympathetic activation of the nervous system that annuls the benefits of the acupuncture interventions.

Overall, from our analysis of the literature, we concluded that positive results were demonstrated when addressing the efficacy of acupuncture treatments to manage PCOS reproductive, metabolic, or mental health features. However, the studies identified in the search did not satisfy all the requirements to be considered high quality research. For that, more high-quality studies are urgent to engage acupuncture as a standardized and scientifically validated technique to support clinical application. In our opinion, the first demand would be to significantly increase the number of RCTs, that will enable a rigorous meta-analysis. We believe that PCOS diagnostic criteria should meet the latest Rotterdam criteria with discrimination of the different PCOS phenotypes [43]. Also, we verified that the design of the acupuncture studies on PCOS was mainly focused on ovarian dysfunction. Hormonal studies contemplated concentrations of FSH and LH, but not any other important biomarkers that would better support the assessment of biochemical hyperandrogenism and hypothalamus-pituitary-gonadal/adrenal dysregulation, such as androstenedione, dehydroepiandrosterone sulfate and sex-hormone-binding globulin.

It is important to point out that our review was limited to literature published in English, with the risk of language bias. For example, 18 of the 178 screened articles were in the Chinese language, and therefore out of the scope of this analysis due to a lack of a valid translation. Also, there was no contact with authors for missing information or data conversions, which might lead to some mis-reading of the data, protocol or even possible misinterpretation of the data discussions.

For a better understanding of the effect of acupuncture on the PCOS condition, in future works, we consider pertinent the development of: 1) more RCTs with improved quality design (with more control groups, especially blank controls) and following STRICTA and/or CONSORT recommendations; 2) more standardized outcomes (whether they would be addressed as primary or secondary); 3) larger samples; 4) whenever possible, double blinded studies; 5) support the rationale behind the design of the study with more references; and 6) referral of the Traditional Chinese Medicine diagnostic with the respective rationale for the general condition of PCOS and the specific symptoms approached in the study design.

DISCLOSURE

Authors declare no financial or non-financial interests re-

lated to the material in the manuscript.

ACKNOWLEDGEMENTS

The authors are grateful to the Biomedical Science Institute Abel Salazar (ICBAS-UP), Porto, on behalf of the Master's Program in TCM, for welcoming and supporting scientific development on knowledge concerning Traditional Chinese Medicine. The authors are also thankful to the Center of Bio-Sciences in Integrative Health (CBSIn), Porto for facilitating the cooperation dynamics in between authors, investigators, and students and for supporting every step of the research performance on the development of Integrative Medicine and Bioscience, from the starting point of the creative process to the final delivery and promotion of the scientific work.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to and approved the final version of the work.

DISCLAIM

This work was developed under the Master Thesis subject to obtain the Master's degree in Traditional Chinese Medicine.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING

This work was not supported by any funding or grants.

ORCID

Natália M. de Oliveira, https://orcid.org/0000-0002-1699-6338
Jorge Machado, https://orcid.org/0000-0003-4570-5445
Lara Lopes, https://orcid.org/0000-0001-6071-0715
Maria Begoña Criado, https://orcid.org/0000-0003-1750-5614

REFERENCES

1. Rasquin Leon LI, Anastasopoulou C, Mayrin JV. Polycystic ovarian disease. In: Abai B, Abu-Ghosh A, Acharya AB, Acharya U, Adhia SG, Sedeh PA, editors. StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.
2. Coutinho EA, Kauffman AS. The role of the brain in the pathogenesis and physiology of polycystic ovary syndrome (PCOS). Med Sci (Basel). 2019;7(8):84.
3. Azziz R. PCOS: a diagnostic challenge. Reprod Biomed Online. 2004;8(6):644-8.
4. Sanchez-Garrido MA, Tena-Sempere M. Metabolic dysfunction in polycystic ovary syndrome: Pathogenic role of androgen excess and potential therapeutic strategies. Mol Metab. 2020;35: 100937.
5. Ho CW, Chen HH, Hsieh MC, Chen CC, Hsu SP, Yip HT, et al. Increased risk of polycystic ovary syndrome and it's comorbidities in women with autoimmune thyroid disease. Int J Environ Res Public Health. 2020;17(7):2422.
6. Aflatounian A, Edwards MC, Rodriguez Paris V, Bertoldo MJ, Desai R, Gilchrist RB, et al. Androgen signaling pathways driving reproductive and metabolic phenotypes in a PCOS mouse model. J Endocrinol 2020;245(3):381-95.
7. Walters KA, Gilchrist RB, Ledger WL, Teede HJ, Handelsman DJ, Campbell RE. New perspectives on the pathogenesis of PCOS: neuroendocrine origins. Trends Endocrinol Metab. 2018;29(12):841-52.
8. Zhang Y, Hu M, Jia W, Liu G, Zhang J, Wang B, et al. Hyperandrogenism and insulin resistance modulate gravid uterine and placental ferroptosis in PCOS-like rats. J Endocrinol. 2020; 246(3):247-63.
9. Cox MJ, Edwards MC, Rodriguez Paris V, Aflatounian A, Ledger WL, Gilchrist RB, et al. Androgen action in adipose tissue and the brain are key mediators in the development of PCOS traits in a mouse model. Endocrinology. 2020;161(7):bqaa061.
10. Brown J, Farquhar C. Clomiphene and other antioestrogens for ovulation induction in polycystic ovarian syndrome. Cochrane Database Syst Rev. 2016;12(12):CD002249.
11. Szilágyi A, Szabó I. Endocrine characteristics of polycystic ovary syndrome (PCOS). Indian J Exp Biol. 2003;41(7):694-700.
12. Pramodh S. Exploration of lifestyle choices, reproductive health knowledge, and polycystic ovary syndrome (PCOS) awareness among female Emirati University students. Int J Womens Health. 2020;12:927-38.
13. Podfigurna A, Meczekalski B, Petraglia F, Luisi S. Clinical, hormonal and metabolic parameters in women with PCOS with different combined oral contraceptives (containing chlormadinone acetate versus drospirenone). J Endocrinol Invest. 2020;43(4):

483-92.
14. Gunning MN, Sir Petermann T, Crisosto N, van Rijn BB, de Wilde MA, Christ JP, et al. Cardiometabolic health in offspring of women with PCOS compared to healthy controls: a systematic review and individual participant data meta-analysis. Hum Reprod Update. 2020;26(1):103-17.
15. Wang H, Ruan X, Li Y, Cheng J, Mueck AO. Oxidative stress indicators in Chinese women with PCOS and correlation with features of metabolic syndrome and dependency on lipid patterns. Arch Gynecol Obstet. 2019;300(5):1413-21.
16. Feichtinger M, Stopp T, Göbl C. [Metabolic and reproductive consequences of the polycystic ovary syndrome (PCOS)]. Wien Med Wochenschr. 2016;166(3-4):139-42. German.
17. Ranasinha S, Joham AE, Norman RJ, Shaw JE, Zoungas S, Boyle J, et al. The association between polycystic ovary syndrome (PCOS) and metabolic syndrome: a statistical modelling approach. Clin Endocrinol (Oxf). 2015;83(6):879-87.
18. Moran LJ, Noakes M, Clifton P, Buckley J, Brinkworth G, Thomson R, et al. Predictors of lifestyle intervention attrition or weight loss success in women with polycystic ovary syndrome who are overweight or obese. Nutrients. 2019;11(3):492.
19. Welt CK, Carmina E. Clinical review: lifecycle of polycystic ovary syndrome (PCOS): from in utero to menopause. J Clin Endocrinol Metab. 2013;98(12):4629-38.
20. van Houten EL, Kramer P, McLuskey A, Karels B, Themmen AP, Visser JA. Reproductive and metabolic phenotype of a mouse model of PCOS. Endocrinology. 2012;153(6):2861-9.
21. Scicchitano P, Dentamaro I, Carbonara R, Bulzis G, Dachille A, Caputo P, et al. Cardiovascular risk in women with PCOS. Int J Endocrinol Metab. 2012;10(4):611-8.
22. Teede HJ, Hutchison S, Zoungas S, Meyer C. Insulin resistance, the metabolic syndrome, diabetes, and cardiovascular disease risk in women with PCOS. Endocrine. 2006;30(1):45-53.
23. Szosland K, Karzkowiak A, Lewinski A. Metabolic characteristics of women diagnosed with polycystic ovary syndrome (PCOS) according to the Rotterdam criteria - our own experience. Neuro Endocrinol Lett. 2019;39(6):434-40.
24. Sachdeva G, Gainder S, Suri V, Sachdeva N, Chopra S. Comparison of the different PCOS phenotypes based on clinical metabolic, and hormonal profile, and their response to clomiphene. Indian J Endocrinol Metab. 2019;23(3):326-31.
25. Kakoly NS, Moran LJ, Teede HJ, Joham AE. Cardiometabolic risks in PCOS: a review of the current state of knowledge. Expert Rev Endocrinol Metab. 2019;14(1):23-33.
26. Patel R, Shah G. High-fat diet exposure from pre-pubertal age induces polycystic ovary syndrome (PCOS) in rats. Reproduction. 2018;155(2):141-51.
27. Torchen LC. Cardiometabolic risk in PCOS: more than a reproductive disorder. Curr Diab Rep. 2017;17(12):137.
28. Kataoka J, Tassone EC, Misso M, Joham AE, Stener-Victorin E, Teede H, et al. Weight management interventions in women with and without PCOS: a systematic review. Nutrients. 2017;9(9):996.
29. Zhang H, Yi M, Zhang Y, Jin H, Zhang W, Yang J, et al. High-fat diets exaggerate endocrine and metabolic phenotypes in a rat model of DHEA-induced PCOS. Reproduction. 2016;151(4):431-41.
30. Sir-Petermann T, Echiburú B, Crisosto N, Maliqueo M, Bravo FP. Metabolic features across the female life span in women with PCOS. Curr Pharm Des. 2016;22(36):5515-25.
31. Barry JA, Qu F, Hardiman PJ. An exploration of the hypothesis that testosterone is implicated in the psychological functioning of women with polycystic ovary syndrome (PCOS). Med Hypotheses. 2018;110:42-5.
32. Jiskoot G, Benneheij SH, Beerthuizen A, de Niet JE, de Klerk C, Timman R, et al. A three-component cognitive behavioural lifestyle program for preconceptional weight-loss in women with polycystic ovary syndrome (PCOS): a protocol for a randomized controlled trial. Reprod Health. 2017;14(1):34.
33. Ilie IR. Neurotransmitter, neuropeptide and gut peptide profile in PCOS-pathways contributing to the pathophysiology, food intake and psychiatric manifestations of PCOS. Adv Clin Chem. 2020;96:85-135.
34. Azizi Kutenaee M, Amirjani S, Asemi Z, Taghavi SA, Allan H, Kamalnadian SN, et al. The impact of depression, self-esteem, and body image on sleep quality in patients with PCOS: a cross-sectional study. Sleep Breath. 2020;24(3):1027-34.
35. Damone AL, Joham AE, Loxton D, Earnest A, Teede HJ, Moran LJ. Depression, anxiety and perceived stress in women with and without PCOS: a community-based study. Psychol Med. 2019;49(9):1510-20.
36. Karjula S, Morin-Papunen L, Auvinen J, Ruokonen A, Puukka K, Franks S, et al. Psychological distress is more prevalent in fertile age and premenopausal women with PCOS symptoms: 15-year follow-up. J Clin Endocrinol Metab. 2017;102(6):1861-9.
37. Williams S, Sheffield D, Knibb RC. 'Everything's from the inside out with PCOS': exploring women's experiences of living with polycystic ovary syndrome and co-morbidities through Skype™ interviews. Health Psychol Open. 2015;2(2):2055102915603051.
38. Bazarganipour F, Ziaei S, Montazeri A, Foroozanfard F, Kazemnejad A, Faghihzadeh S. Health-related quality of life in patients with polycystic ovary syndrome (PCOS): a model-based study of predictive factors. J Sex Med. 2014;11(4):1023-32.
39. Dokras A. Mood and anxiety disorders in women with PCOS. Steroids. 2012;77(4):338-41.
40. Mannerås L, Cajander S, Lönn M, Stener-Victorin E. Acupunc-

ture and exercise restore adipose tissue expression of sympathetic markers and improve ovarian morphology in rats with dihydrotestosterone-induced PCOS. Am J Physiol Regul Integr Comp Physiol. 2009;296(4):R1124-31.
41. Witchel SF, Teede HJ, Peña AS. Curtailing PCOS. Pediatr Res. 2020;87(2):353-61.
42. Teede HJ, Misso ML, Boyle JA, Garad RM, McAllister V, Downes L, et al.; International PCOS Network. Translation and implementation of the Australian-led PCOS guideline: clinical summary and translation resources from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Med J Aust. 2018;209(S7):S3-8.
43. Azziz R, Kintziger K, Li R, Laven J, Morin-Papunen L, Merkin SS, et al. Recommendations for epidemiologic and phenotypic research in polycystic ovary syndrome: an androgen excess and PCOS society resource. Hum Reprod. 2019;34(11):2254-65.
44. Lie Fong S, Douma A, Verhaeghe J. Implementing the international evidence-based guideline of assessment and management of polycystic ovary syndrome (PCOS): how to achieve weight loss in overweight and obese women with PCOS? J Gynecol Obstet Hum Reprod. 2021;50(6):101894.
45. Ferreira AS, Luiz AB. Role of dermatomes in the determination of therapeutic characteristics of channel acupoints: a similarity-based analysis of data compiled from literature. Chin Med. 2013;8:24.
46. Ee C, Smith CA, Costello M, Moran L, Steiner GZ, Stepto N, et al. Acupuncture or auricular electro-acupuncture as adjuncts to lifestyle interventions for weight management in PCOS: protocol for a randomised controlled feasibility study. Pilot Feasibility Stud. 2020;6:53.
47. Zhou F, Huang D, YingXia. Neuroanatomic basis of acupuncture points: a neurobiological view. In: Xia Y, Cao X, Wu G, Cheng J, editors. Acupuncture therapy for neurological diseases. Berlin: Springer; 2010. p. 32-80.
48. Lim CED, Wong FWS, Smith W. Factors influencing the bioimpedance data in tissue segments along the three arm meridians: a pilot study. CellMed. 2011;1(1):e7.
49. Sun J, Jin C, Wu H, Zhao J, Cui Y, Liu H, et al. Effects of electroacupuncture on ovarian P450arom, P450c17α and mRNA expression induced by letrozole in PCOS rats. PLoS One. 2013;8(11):e79382.
50. Li J, Cui W, Sun W, Zhang QY, Guan Q. [Effect of electroacupuncture on the spindle and oocytes quality in patients with PCOS]. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2015;35(3):304-9. Chinese.
51. Stener-Victorin E, Maliqueo M, Soligo M, Protto V, Manni L, Jerlhag E, et al. Changes in HbA1c and circulating and adipose tissue androgen levels in overweight-obese women with polycystic ovary syndrome in response to electroacupuncture. Obes Sci Pract. 2016;2(4):426-35.
52. Xu G, Zhang A, Liu J, Wang X, Feng J, Chen Y. Effects of electroacupuncture on ovarian expression of the androgen receptor and connexin 43 in rats with letrozole-induced polycystic ovaries. Evid Based Complement Alternat Med. 2020;2020:3608062.
53. Feng Y, Johansson J, Shao R, Mannerås-Holm L, Billig H, Stener-Victorin E. Electrical and manual acupuncture stimulation affect oestrous cyclicity and neuroendocrine function in an 5α-dihydrotestosterone-induced rat polycystic ovary syndrome model. Exp Physiol. 2012;97(5):651-62.
54. Xu L, Qiao X. Acupuncture is not as effective as infertility treatment in women with PCOS. Evid Based Med. 2017;22(6):229-30.
55. Wang Q, Deng H, Cheng K, Huang Z, Yin X, Zhou Y, et al. Manual acupuncture for the infertile female with polycystic ovary syndrome (PCOS): study protocol for a randomized sham-controlled trial. Trials. 2019;20(1):564.
56. Zhang HL, Huo ZJ, Wang HN, Wang W, Chang CQ, Shi L, et al. [Acupuncture ameliorates negative emotion in PCOS patients: a randomized controlled trial]. Zhongguo Zhen Jiu. 2020;40(4):385-90. Chinese.
57. Stener-Victorin E, Lundeberg T, Waldenström U, Manni L, Aloe L, Gunnarsson S, et al. Effects of electro-acupuncture on nerve growth factor and ovarian morphology in rats with experimentally induced polycystic ovaries. Biol Reprod. 2000;63(5):1497-503.
58. Manni L, Lundeberg T, Holmäng A, Aloe L, Stener-Victorin E. Effect of electro-acupuncture on ovarian expression of alpha (1)- and beta (2)-adrenoceptors, and p75 neurotrophin receptors in rats with steroid-induced polycystic ovaries. Reprod Biol Endocrinol. 2005;3:21.
59. Hickman FE, Stanley EM, Carter BD. Neurotrophin responsiveness of sympathetic neurons is regulated by rapid mobilization of the p75 receptor to the cell surface through TrkA activation of Arf6. J Neurosci. 2018;38(24):5606-19.
60. Stener-Victorin E, Jedel E, Mannerås L. Acupuncture in polycystic ovary syndrome: current experimental and clinical evidence. J Neuroendocrinol. 2008;20(3):290-8.
61. Xiang S, Xia MF, Song JY, Liu DQ, Lian F. Effect of electroacupuncture on expression of IRS-1/PI3K/GLUT4 pathway in ovarian granulosa cells of infertile patients with polycystic ovary syndrome-insulin resistance of Phlegm-Dampness syndrome. Chin J Integr Med. 2021;27(5):330-5.
62. Wu J, Chen X. Acupuncture therapy protects PCOS patients with diabetes by regulating miR-32-3p/PLA2G4A pathway. Am J Transl Res. 2021;13(8):8819-32.
63. Zhao QY, Sun Y, Zhou J, Gao YL, Ma GZ, Hu ZH, et al. Effec-

tiveness of herb-partitioned moxibustion combined with electroacupuncture on polycystic ovary syndrome in patients with symptom pattern of kidney deficiency and phlegm-dampne. J Tradit Chin Med. 2021;41(6):985-93.
64. Shi Y, Li L, Zhou J, Sun J, Chen L, Zhao J, et al. Efficacy of electroacupuncture in regulating the imbalance of AMH and FSH to improve follicle development and hyperandrogenism in PCOS rats. Biomed Pharmacother. 2019;113:108687.
65. Tong X, Liu Y, Xu X, Shi J, Hu W, Ma T, et al. Ovarian innervation coupling with vascularity: the role of electro-acupuncture in follicular maturation in a rat model of polycystic ovary syndrome. Front Physiol. 2020;11:474.
66. Peng Y, Guo L, Gu A, Shi B, Ren Y, Cong J, et al. Electroacupuncture alleviates polycystic ovary syndrome-like symptoms through improving insulin resistance, mitochondrial dysfunction, and endoplasmic reticulum stress via enhancing autophagy in rats. Mol Med. 2020;26(1):73.
67. Manneråas L, Jonsdottir IH, Holmäng A, Lönn M, Stener-Victorin E. Low-frequency electro-acupuncture and physical exercise improve metabolic disturbances and modulate gene expression in adipose tissue in rats with dihydrotestosterone-induced polycystic ovary syndrome. Endocrinology. 2008;149(7):3559-68.
68. Peng Y, Yang X, Luo X, Liu C, Cao X, Wang H, et al. Novel mechanisms underlying anti-polycystic ovary like syndrome effects of electroacupuncture in rats: suppressing SREBP1 to mitigate insulin resistance, mitochondrial dysfunction and oxidative stress. Biol Res. 2020;53(1):50.
69. Johansson J, Manneråas-Holm L, Shao R, Olsson A, Lönn M, Billig H, et al. Electrical vs manual acupuncture stimulation in a rat model of polycystic ovary syndrome: different effects on muscle and fat tissue insulin signaling. PLoS One. 2013;8(1):e54357.
70. Chen SZ. [An analysis on correlation between acupoint and target organs of acupuncture-moxibustion from the perspective of modern medical sciences]. Zhen Ci Yan Jiu. 2019;44(8):620-4. Chinese.
71. Chen H, Lim CED. The efficacy of using acupuncture in managing polycystic ovarian syndrome. Curr Opin Obstet Gynecol. 2019;31(6):428-32.
72. Yang ES, Li PW, Nilius B, Li G. Ancient Chinese medicine and mechanistic evidence of acupuncture physiology. Pflugers Arch. 2011;462(5):645-53.
73. Lim CED, Ng RWC, Cheng NCL, Zhang GS, Chen H. Acupuncture for polycystic ovarian syndrome. Cochrane Database Syst Rev. 2019;7(7):CD007689.
74. Lim CE, Wong WS. Current evidence of acupuncture on polycystic ovarian syndrome. Gynecol Endocrinol. 2010;26(6):473-8.
75. Amir-Behghadami M, Janati A. Population, intervention, comparison, outcomes and study (PICOS) design as a framework to formulate eligibility criteria in systematic reviews. Emerg Med J. 2020;37(6):387.
76. Banting LK, Gibson-Helm M, Polman R, Teede HJ, Stepto NK. Physical activity and mental health in women with polycystic ovary syndrome. BMC Womens Health. 2014;14(1):51.
77. El-Mekawy HS, ElDeeb AM, Ghareib HO. Effect of laser acupuncture combined with a diet-exercise intervention on metabolic syndrome in post-menopausal women. J Adv Res. 2015;6(5):757-63.
78. Hong M, Xiaohong Q, Cai W, Xiuhua C. Pcos-induced anxiety disorder treated mainly with acupuncture therapy: a randomized controlled trial. Acta Med Mediterr. 2016;32:1155-8.
79. Wu XK, Stener-Victorin E, Kuang HY, Ma HL, Gao JS, Xie LZ, et al.; PCOSAct Study Group. Effect of acupuncture and clomiphene in Chinese women with polycystic ovary syndrome: a randomized clinical trial. JAMA. 2017;317(24):2502-14.
80. El-Shamy FF, El-Kholy SS, El-Rahman MMA. Effectiveness of laser acupoints on women with polycystic ovarian syndrome: a randomized controlled trial. J Lasers Med Sci. 2018;9(2):113-20.
81. Cao Y, Chen H, Zhao D, Zhang L, Yu X, Zhou X, et al. The efficacy of Tung's acupuncture for sex hormones in polycystic ovary syndrome: a randomized controlled trial. Complement Ther Med. 2019;44:182-8.
82. Wang Z, Dong H, Wang Q, Zhang L, Wu X, Zhou Z, et al. Effects of electroacupuncture on anxiety and depression in unmarried patients with polycystic ovarian syndrome: secondary analysis of a pilot randomised controlled trial. Acupunct Med. 2019;37(1):40-6.
83. Pundir J, Charles D, Sabatini L, Hiam D, Jitpiriyaroj S, Teede H, et al. Overview of systematic reviews of non-pharmacological interventions in women with polycystic ovary syndrome. Hum Reprod Update. 2019;25(2):243-56.
84. Hsu MI. Changes in the PCOS phenotype with age. Steroids. 2013;78(8):761-6.
85. MacPherson H, Altman DG, Hammerschlag R, Youping L, Taixiang W, White A, et al. Revised standards for reporting interventions in clinical trials of acupuncture (STRICTA): extending the CONSORT statement. Acupunct Relat Ther. 2015;3(4):35-46.
86. Bian Z, Liu B, Moher D, Wu T, Li Y, Shang H, et al. Consolidated standards of reporting trials (CONSORT) for traditional Chinese medicine: current situation and future development. Front Med. 2011;5(2):171-7.
87. Torgerson DJ, Torgerson CJ. Designing randomised trials in health, education and the social sciences: an introduction. London: Palgrave Macmillan; 2008.
88. Li R, Yu G, Yang D, Li S, Lu S, Wu X, et al. Prevalence and predictors of metabolic abnormalities in Chinese women with

PCOS: a cross- sectional study. BMC Endocr Disord. 2014;14:76.
89. Hosseinpanah F, Barzin M, Keihani S, Ramezani Tehrani F, Azizi F. Metabolic aspects of different phenotypes of polycystic ovary syndrome: Iranian PCOS prevalence study. Clin Endocrinol (Oxf). 2014;81(1):93-9.
90. Glintborg D, Mumm H, Hougaard D, Ravn P, Andersen M. Ethnic differences in Rotterdam criteria and metabolic risk factors in a multiethnic group of women with PCOS studied in Denmark. Clin Endocrinol (Oxf). 2010;73(6):732-8.
91. Williams S, Sheffield D, Knibb RC. The polycystic ovary syndrome quality of life scale (PCOSQOL): development and preliminary validation. Health Psychol Open. 2018;5(2):2055102918788195.