PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Auricular points acupressure for insulin resistance in overweight/obese women with polycystic ovary syndrome: protocol for a randomized controlled pilot trial
AUTHORS	Li, Yan; Hou, Lihui; Wang, Yingji; Xie, Liangzhen; Zhang, Meiwei; Pan, Zimeng; Li, Yangyang; Ding, Yicheng; Sun, Miao; Qu, Yufang; Liu, Songjiang

VERSION 1 - REVIEW

REVIEWER	Shifen Xu Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai, China
REVIEW RETURNED	15-Nov-2018

GENERAL COMMENTS	This is a simple clinical trial protocol of auricular points acupressure for women with PCOS to lose weight. The study has some limitations in the design and lots of grammar problems in the manuscript. 1. It seems confusing about the relationship between the Abstract part and the title. The trial studies about the effect of AA for PCOS women to lose weight according to the title. But author spent too much on explaining PCOS in the text, not on the obesity problem. 2. This is a double-blinded randomized trial, which means the acupuncturist will also be blind to the group assignment. But I haven't seen any related procedures about how to implement the double-blinded method, even in the Blinding part. 3. Participants in both groups will do acupressure on the different auricular points, with same frequency and seek for soreness or heat feeling. The results can be explained by the specificity of the auricular acupoints but not for the effect of the acupressure. Besides, this kind of intervention can't be regarded as "sham AA". 4. In the Allocation concealment part (Page 11, line 4), there should be more details. For example, is there any independent investigator charge of this procedure? And "the sealed envelopes will only be opened" by whom? 5. In the Inclusion criteria part, I can't understand the third criterion " 2 years after menarche". 6. In the Sample size calculation part, the sample size calculation method of this trial didn't match the reference 31. 7. There was no timepoint of "informed consent" in Table 1, and no illustration of t0 or illustration for "baseline features". Besides, I

	wonder whether there was an interval between baseline assessment and the intervention period and why. 8. The flowchart of the study (Figure 1) is relatively simple and hard to understand. 9. Page 3, line 22, I guess the author might like to say “in both physical and psychological well-being”. Some similar mistakes can be also seen on page 9 line 40, “replaced by the by the” . There are also lots of grammar mistakes and Chinglish expression in this manuscript. Authors need to check the manuscript carefully before submitting.

REVIEWER	Renato Pasquali University of Bologna
REVIEW RETURNED	27-Nov-2018

GENERAL COMMENTS	This paper presents a randomized, placebo controlled pilot trial to evaluate the safety and effectiveness of auricular point acupressure in women with PCOS. No data are presented. Obviously, the acceptance should be by the Editor (note that I do not know if the journal accepts projects like this) I apologize. I recommended ""reject" but it could be I'm wrong

REVIEWER	Ernest HY Ng The University of Hong Kong, HKSAR, China
REVIEW RETURNED	30-Nov-2018

GENERAL COMMENTS	The aim of this randomized placebo controlled trial is to study to investigate the effectiveness of auricular acupuncture on insulin sensitivity in women with PCOS. Strength of the study 1. This is a randomized placebo controlled trial. Major concerns 1. Introduction • The authors can give a summary of acupuncture on insulin sensitivity in PCOS women, although there is no study on auricular acupuncture. • Page 4 Line 48: The following statement “It is easily accepted by patients since its effectiveness and non-invasion.” is not supported by evidence. The aim of the present study is to find out auricular acupuncture can have effect on insulin sensitivity. • References 8-11 mainly studied the weight change using auricular acupuncture. On the other hand, the authors investigated insulin sensitivity as the primary outcome parameter. They failed to highlight the significance of insulin sensitivity in clinical practice. 2. Materials and methods • Page 8 Line 17-20: How can the researchers be certain those acupoints chosen in the placebo group had no treatment effects on PCOS women. Any evidence to support the choice? • Page 8 Line 33-35: Women are requested to apply massage over the magnetic beads applied four times a day. How can the researchers ensure or measure the compliance especially the study would last for 12 weeks.

	• Page 9: Anxiety and stress were not measured despite they are stated to be important in the Introduction. • Page 9 Line 27: sample size calculation can be based on some assumption of the changes in the primary outcome parameter. Minor concerns: Title Suggest to add the primary outcome parameter to make it more clear Materials and methods Page 5 Line 4: Diagnostic criteria of PCOS has been recently updated. Page 6 Line 12: add the primary outcome parameter after effectiveness

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Shifen Xu

Institution and Country: Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai, China

Please state any competing interests or state ‘None declared’: None declared.

Please leave your comments for the authors below

This is a simple clinical trial protocol of auricular points acupressure for women with PCOS to lose weight. The study has some limitations in the design and lots of grammar problems in the manuscript.

1.It seems confusing about the relationship between the Abstract part and the title. The trial studies about the effect of AA for PCOS women to lose weight according to the title. But author spent too much on explaining PCOS in the text, not on the obesity problem.

Response:

The primary outcome of the present trial is changes in HOMA-IR between baseline and after 3 months of AA/sham AA treatment within PCOS patients, which indicates insulin resistance (IR). Although its exact etiology remains elusive, PCOS is known to feature several hormonal disturbances, including hyperandrogenemia, IR, and hyperinsulinemia. Menstrual irregularities and anovulation appear to be more prevalent and severe in obese women with PCOS than in their non-obese counterparts, and weight loss of at least 5% tends to be associated with improvement of these conditions. Based on previous literature, AA is effective in weight loss so it may be an effective treatment for PCOS as well. Since the close association between IR and obesity, the present trial chose HOMA-IR as the primary outcome. To avoid further confusion, the authors have changed the title following the editor and the reviewers’ advice.

2. This is a double-blinded randomized trial, which means the acupuncturist will also be blind to the group assignment. But I haven’t seen any related procedures about how to implement the double-blinded method, even in the Blinding part.

Response:

We appreciated the reviewer for the notice. Patients, investigators, and outcome assessors will be blinded to the AA treatment assignment but not the acupuncturists. The author also deleted the “double blind” term in the revised manuscript.

3. Participants in both groups will do acupressure on the different auricular points, with same frequency and seek for soreness or heat feeling. The results can be explained by the specificity of the auricular acupoints but not for the effect of the acupressure. Besides, this kind of intervention can’t be regarded as “sham AA”.

Response:

We appreciated the suggestions of the reviewer at this point. Acupressure [from Latin acus "needle" (see acuity) + pressure (n.)] is an alternative medicine technique similar in principle to acupuncture. In treatment, physical pressure is applied to acupuncture points with the aim of clearing blockages in these meridians. Pressure may be applied by hand, by elbow, or with various devices. (https://en.wikipedia.org/wiki/Acupressure)

As the definition of acupressure, the effects of acupressure should differ from different acupoints. By “sham AA”, the authors refer to “sham auricular points acupressure”. The active points of the present trial were chosen based on published studies and the sham acupoints were chosen because they have no therapeutic effects in treating PCOS or PCOS related conditions as recorded. The purpose of the present trials is to investigate the effect of the auricular acupoints rather than the “pressure”.

4. In the Allocation concealment part (Page 11, line 4), there should be more details. For example, is there any independent investigator charge of this procedure? And “the sealed envelopes will only be opened” by whom?

Response:

We appreciated the reviewer’s suggestion. The procedure was conducted by an independent agency, that personnel will not be involved in the implementation process. After the investigator had obtained the patient’s consent, he/she will get the assignment information from the above-mentioned agency and put the participant’s name and other details on the envelope. Then the envelope will be opened directly by the acupuncturist who provided AA treatment after the subject has completed baseline clinical assessments. We’ve detailed this part in the manuscript as the reviewer suggested.

5. In the Inclusion criteria part, I can’t understand the third criterion “2 years after menarche”.

Response:

This is because the present trial was not excluded adolescent PCOS subject, however, to diagnose an adolescent PCOS, 2 years after menarche is recommended by guidelines.

6. In the Sample size calculation part, the sample size calculation method of this trial didn’t match the reference 31.

Response:

Please refer to Page 15-item-7a-explanation in reference 31 (which was renumbered as 35 in revision). Since the present trial was designed as a pilot study, the investigators employed a small standardised effect size. Each treatment arm is set to 25 subjects, with an estimated 20% drop out rate, and the recruitment plan was 30 subjects per group.

7. There was no timepoint of “informed consent” in Table 1, and no illustration of t0 or illustration for “baseline features”. Besides, I wonder whether there was an interval between baseline assessment and the intervention period and why.

Response:

We have detailed information as below and in the manuscript. Informed consent was obtained during enrollment. Baseline features: HOMA-IR (primary outcome), hormonal profile including: testosterone (T), androstadienedione (AND), sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), follicle stimulating hormone (FSH), luteinizing hormone(LH), estradiol (E2), weight, waist/hip circumference, BMI, blood pressure, FG score and acne. T0 referred to enrollment. There was no interval between baseline assessment and intervention because we exclude subjects taken medications known to affect reproductive function or metabolism within the past three months.

8. The flowchart of the study (Figure 1) is relatively simple and hard to understand.

Response:

Flowchart was updated.

9. Page 3, line 22, I guess the author might like to say “in both physical and psychological well-being”. Some similar mistakes can be also seen on page 9 line 40, “replaced by the by the” . There are also lots of grammar mistakes and Chinglish expression in this manuscript. Authors need to check the manuscript carefully before submitting.

Response:

We appreciated the reviewer for reading the manuscript thoroughly and apologize for the typo and mistakes in writing. We have re-edited the manuscript.

Reviewer: 2

Reviewer Name: Renato Pasquali

Institution and Country: University of Bologna

Please state any competing interests or state ‘None declared’: No conflict of interest

Please leave your comments for the authors below

This paper presents a randomized, placebo controlled pilot trial to evaluate the safety and effectiveness of auricular point acupressure in women with PCOS. No data are presented.

Obviously, the acceptance should be by the Editor (note that I do not know if the journal accepts projects like this) I apologize. I recommended ""reject" but it could be I'm wrong

Reviewer: 3

Reviewer Name: Ernest HY Ng

Institution and Country: The University of Hong Kong, HKSAR, China

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

The aim of this randomized placebo controlled trial is to study to investigate the effectiveness of auricular acupuncture on insulin sensitivity in women with PCOS.

Strength of the study

1. This is a randomized placebo controlled trial.

Major concerns

1. Introduction

- The authors can give a summary of acupuncture on insulin sensitivity in PCOS women, although there is no study on auricular acupuncture.

Response:

The author had added the summary as suggested.

- Page 4 Line 48: The following statement “It is easily accepted by patients since its effectiveness and non-invasion.” is not supported by evidence. The aim of the present study is to find out auricular acupuncture can have effect on insulin sensitivity.

Response:

The sentence has been deleted.

- References 8-11 mainly studied the weight change using auricular acupuncture. On the other hand, the authors investigated insulin sensitivity as the primary outcome parameter. They failed to highlight the significance of insulin sensitivity in clinical practice.

Response:

The author downsized this part.

2. Materials and methods

- Page 8 Line 17-20: How can the researchers be certain those acupoints chosen in the placebo group had no treatment effects on PCOS women. Any evidence to support the choice?

Response:

There is no clinical report indicating acupoints in the placebo group have no treatment effects on PCOS for now. The author chose the acupoints based on their effects recorded in textbooks (Wang FC, Ma TM. Method of acupuncture and moxibustion (Fourth edition) [M]. China: China Press of Traditional Chinese Medicine, 2016: 97,100-101). LO5 (Yan) is recorded to treat eye diseases such as pseudo-myopia, Red & Bloodshot Eyes. LO7 (Bian tao ti) is used to treat tonsillitis and pharyngitis. SF3 (Zhou) is used to treat external supracondylar of humerus arthritis and elbow pain. We appreciated the reviewer’s suggestion and the reference was also added in the manuscript.

- Page 8 Line 33-35: Women are requested to apply massage over the magnetic beads applied four times a day. How can the researchers ensure or measure the compliance especially the study would last for 12 weeks.

Response:

Adherence to message over the magnetic beads was tracked via self-report logs, which was obtained at the weekly frequency during AA/sham AA treatment. This was added to the intervention section of the manuscript.

- Page 9: Anxiety and stress were not measured despite they are stated to be important in the Introduction.

Response:

The present trial did not assess anxiety and depression using specific questionnaires. The author re-edit the manuscript focused on the quality of life aspects which were measured by the questionnaires applied in the trial.

- Page 9 Line 27: sample size calculation can be based on some assumption of the changes in the primary outcome parameter.

Response:

While initialed the trial, investigators calculated sample size as the reviewer suggested, it turned out to be a much larger sample size than the current one. Considering the feasibility and the budget of the program, investigators started with this pilot trial using the smaller sample size recommended by literature. The result of this trial will provide data to calculated sample size for the future RCT.

Minor concerns:

Title

- Suggest to add the primary outcome parameter to make it more clear.

Response:

The title has changed to “Auricular points acupressure for insulin resistance of overweight/obese women with polycystic ovary syndrome: protocol for a randomized controlled pilot trial”.

Materials and methods

- Page 5 Line 4: Diagnostic criteria of PCOS has been recently updated.

Response:

We appreciated the reminder of the reviewer and looked through the “International evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018”. However, the trial currently has recruited more than 3 quarters of planned subjects, after discussed with all investigators, we will not change the diagnostic criteria of PCOS in the ongoing trial, but will use the updated one in the future trial designed based on the result of the present pilot trial.

- Page 6 Line 12: add the primary outcome parameter after effectiveness

Response:

We added the primary outcome parameter followed the reviewer’s comments.

VERSION 2 – REVIEW

REVIEWER	Shifen Xu Shanghai Municipal Hospital of Traditional Chinese Medicine
REVIEW RETURNED	16-Jan-2019

GENERAL COMMENTS	Though the authors tried to make some changes for the manuscript, I'm sorry but I still suggest "reject" for this protocol with the following reasons: 1. In the "introduction" part, the author added many related RCT about acupuncture for PCOS which seemed very confusing; because the effects of pressing auricular acupoints was totally different from those of acupuncture for treating disease. I can't understand why the author did such analogy. 2. There were still many spelling or grammatical mistakes in the revised paper, for example, the Interventions part, line3, "the senier acupuncture" should be "senior"; and the "Randomization" part, line5, "Personal involved" should be "person" etc. Authors must be very careful before uploading the paper. 3. There was no related information about the blind method in the paper, so how to guarantee that other researchers except the acupuncturist are blinded to the group assignment? 4. How and when do the researchers make the outcome assessment? I found no details in the paper. 5. I wonder why there was no "discussion" part in the paper.

REVIEWER	Professor Ernest HY Ng The University of Hong Kong, Hong Kong SAR
REVIEW RETURNED	13-Jan-2019

GENERAL COMMENTS	The sample size calculation is not complete in the text and the authors should consult a statistician to calculate the sample size required for the primary outcome.

VERSION 2 – AUTHOR RESPONSE

Reviewer Name: Professor Ernest HY Ng

Comments: The sample size calculation is not complete in the text and the authors should consult a statistician to calculate the sample size required for the primary outcome.

Answer: Thanks for your suggestion. This trial is a pilot study. Now there is few trials concerning AA on insulin sensitivity in PCOS women and a pilot study is an important step in the assessment of an intervention. So we hope to conduct a pilot study for providing information to design the future definitive AA trial. Traditional power calculations are inappropriate for pilot studies, although sample size justification is important. Based on CONSORT 2010 statement (extension to randomized pilot and feasibility trials), sample size calculation is not exactly performed in this pilot trial, since the key objective of this pilot trials focuses on the acceptability or feasibility of introducing AA on IR in women with PCOS.

Reviewer Name: Shifen Xu

Comments:1. In the "introduction" part, the author added many related RCT about acupuncture for PCOS which seemed very confusing; because the effects of pressing auricular acupoints was totally different from those of acupuncture for treating disease. I can't understand why the author did such analogy.

Answer: A summary of acupuncture on insulin sensitivity in PCOS women was suggested by the other reviewer, the author agreed with his opinion and added this part. In clinical practice, AA and acupuncture are considered as different treatments, however there is no evidence indicates they have totally different effect and mechanism in treating PCOS or IR. In Chinese Medicine theory, AA is also considered as part of acupuncture. So the author would like to keep this part in the manuscript.

Comments:2. There were still many spelling or grammatical mistakes in the revised paper, for example, the Interventions part, line3, "the senier acupuncture" should be "senior"; and the "Randomization" part, line5, "Personal involved" should be "person" etc. Authors must be very careful before uploading the paper.

Answer: The author invited a native English speaking personnel to proof reading the manuscript and made corrections.

Comments:3. There was no related information about the blind method in the paper, so how to guarantee that other researchers except the acupuncturist are blinded to the group assignment?

Answer: The way to keep other researchers blinded to the group assignment were partly mentioned in the allocation concealment part and the author also detailed the blinding part as suggested. After the acupuncturist open the envelope and got the assignment information, he/she were instructed not to exchange or reveal assignment information to other investigators or participants. Outcome assessors and data analysts were blind to grouping or treatment. Participants were arranged in different time schedule to avoid met with each other and information exchange. The assignment information will not be revealed until the end of the study, unless there is a serious adverse event.

Comments: 4. How and when do the researchers make the outcome assessment? I found no details in the paper.

Answer: Outcome measures were collected at baseline and at the end of treatments visit as showed in table 1. "Schedule of enrollment, interventions and assessments". The primary outcome of the present trial is "changes in HOMA-IR between baseline and after 3 months of AA/sham AA treatment", the calculation method is provided in the "Inclusion criteria" part where the term HOMA-IR first appeared in the manuscript. Fasting blood sample for hormonal profile assessment listed in secondary outcome was obtained at baseline and at the end of treatments. Body composition including Weight, waist/hip circumference, BMI, blood pressure, FG score, and acne lesion counts will be done at baseline and at the end of treatments visit by outcome assessors. Health-related quality-of-life was assessed through the questionnaires listed, which obtained from the subjects at baseline and at the end of treatments. Adverse events will be collected during the weekly treatment visits or through phone calls. The author had detailed the manuscript as suggested.

Comments: 5. I wonder why there was no "discussion" part in the paper.

Answer: The present manuscript followed the instruction of submission guidelines of BMJ Open (study protocol), and discussion is not a must-have item listed in the guideline for protocol. Since the present manuscript is just the protocol of a clinical trial, the authors thought discussion was not a necessity in the protocol so there was no discussion in the manuscript.

VERSION 3 – REVIEW

REVIEWER	Shifen Xu Shanghai Municipal Hospital of Traditional Chinese Medicine
REVIEW RETURNED	24-Feb-2019

GENERAL COMMENTS	I noticed that authors did lots of corrections in the present manuscript and all my questiones were properly answered. Good luck.

REVIEWER	Ernest HY Ng The University of Hong Kong, HKSAR
REVIEW RETURNED	24-Feb-2019

GENERAL COMMENTS	The sample size calculation is not clear enough for a pilot randomized trial. The authors should state more clearly the chosen sample size as follow. "Most methodological papers that focus on recommendations about sample size requirements for pilot trials assume that the main aim of such a trial is to estimate a quantitative measure such as the variance (or standard deviation) of an effect size to inform the sample sample size calculation for a future definitive RCT. Methods focus on the precision with which such estimates can be obtained. Whitehead et al suggests that the size of a pilot trial should be related to the size of the future definitive RCT. For such a trial designed with 90% power and two sided 5% significance, they recommend pilot trial sample sizes for each treatment arm of 75, 25, 15, and 10 for standardised effect sizes that are extra small (0.1), small (0.2), medium (0.5), or large (0.8), respectively.' Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. Stat Methods Med Res 2016;25:1057-73.

VERSION 3 – AUTHOR RESPONSE

Reviewer Name: Professor Ernest HY Ng

Comments: The sample size calculation is not clear enough for a pilot randomized trial. The authors should state more clearly the chosen sample size as follow.

"Most methodological papers that focus on recommendations about sample size requirements for pilot trials assume that the main aim of such a trial is to estimate a quantitative measure such as the variance (or standard deviation) of an effect size to inform the sample sample size calculation for a

future definitive RCT. Methods focus on the precision with which such estimates can be obtained. Whitehead et al suggests that the size of a pilot trial should be related to the size of the future definitive RCT. For such a trial designed with 90% power and two sided 5% significance, they recommend pilot trial sample sizes for each treatment arm of 75, 25, 15, and 10 for standardised effect sizes that are extra small (0.1), small (0.2), medium (0.5), or large (0.8), respectively. "

Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. Stat Methods Med Res 2016;25:1057-73.

Answer: We appreciated the reviewer's suggestion and revised the manuscript.