Author’s response to reviews

Title: A three-arm, multicenter, open-label randomized controlled trial of hydroxychloroquine and low-dose prednisone to treat recurrent pregnancy loss in women with undifferentiated connective tissue diseases: Protocol for the immunosuppressant regimens for Living FEtuses (ILIFE) Trial

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Cover Letter

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A three-arm, multicenter, open-label randomized controlled trial of hydroxychloroquine and low-dose prednisone on recurrent spontaneous abortion with undifferentiated connective tissue diseases: Protocol for the immunosuppressant regimens for Living FEtuses (ILIFE) Trial
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Dear Prof. Ines Rombach,

Thank you very much for sending the reviewers’ comments on our manuscript “A three-arm, multicenter, open-label randomized controlled trial of hydroxychloroquine and low-dose prednisone on recurrent spontaneous abortion with undifferentiated connective tissue diseases: Protocol for the immunosuppressant regimens for Living FEtuses (ILIFE) Trial”.


We thank the reviewers for their constructive comments that has helped us to improve the manuscript. We have revised the manuscript accordingly. Below is the itemized responses to the comments. We hope the manuscript is now acceptable to be published on Trials.

Yours sincerely,

Liangjing Lu

Author Response

Response to Reviewer 1: Prof. Jane Daniels

Comment 1: Introduction: Would consider using the term recurrent miscarriage instead of recurrent spontaneous abortion throughout. The latter is preferred term in journals such as BJOG and AJOG, and by many women. Similarly, please don't refer to "RSA women" - describe the population as "women who have had recurrent miscarriages".

Response: Thank you for this comment and we have change the description on the population (Page 5, Line 102). There is no consensus on the term. Recurrent miscarriage (RM), recurrent spontaneous abortion (RSA), recurrent pregnancy loss (RPL) are commonly used to describe abortion in different countries. RPL is used by American Society for Reproductive Medicine (ASRM), and RM by Royal College of Obstetricians and Gynaecologists (RCOG). RSA is the term used by others ((Arch Gynecol Obstet. 2005 Jul;272(2):95-108. DOI: 10.1007/s00404-004-0706-y; Iran J Pathol. 2020 Winter;15(1):19-22. doi: 10.30699/IJP.2019.102943.2026) and in China. If possible, we prefer to use RSA in order to comply with the naming convention in China.

Comment 2: Please refer to systematic reviews eg Cochrane Database Syst Rev. 2014 Jul 4;(7); Medicine (Baltimore). 2015 Nov;94(45):e1732. ; RMD Open. 2019 Apr 28;5(1):e000924 or guidelines eg Hum Reprod Open. 2018 Apr 6;2018(2):hoy004. instead of individual trials, cite the treatment effect and grade of evidence and reflect on the anticipate treatment effect in women with UCTD compared to CTD or APS.

Response: Thanks for the helpful comments. We have researched some systematic reviews and guidelines and added a summary to the introduction section (page 5-6 line 108-136). In 2016 guideline on prescribing drugs in pregnancy and breastfeeding, corticosteroids and HCQ is compatible with each trimester of pregnancy [level of evidence 1++, grade of recommendation A, strength of agreement 100%]. But the treatment effect on pregnancy outcomes of UCTD women was poorly studied and the sample size of previous studies was small. The studies of prednisone and HCQ in the treatment of recurrent miscarriage, SLE and APS showed that they could reduce the incidence of disease flares and obstetric complications. The impact on pregnancy outcome of UCTD is probably less severe than well-established rheumatic diseases, and the therapeutic effect may be weaker. However, the prevalence of UCTD is high, which needs to be paid attention to.
Comment 3: Methods
How are previous miscarriages verified- from reports from women or only those managed in a healthcare facility? Are there criteria around the gestational age/ trimester at which the miscarriage occurred?

Response: Thanks for the comment. We have revised the Materials and Methods section with more details (page 8 line 169). Previous miscarriages verified from both reports from women and those managed in the hospital. The miscarriage was defined as pregnancy loss occurred in all trimesters.

Comment 4: The requirement for the presence of at least one autoantibody- is there a complete list of qualifying autoantibodies? Are there any thresholds for positivity or is the normal reference range for each hospital? If so, do these reference ranges apply to young women?

Response: We greatly appreciate the comment. Yes, there is a complete list of qualifying autoantibodies, including ANA, ENA, ds-DNA, aPL, ACA, Anti-CCP, et al. There is normal reference range for each hospital according the laboratory. And there is no difference in ANA and other antibody tests regarding young women population.

Comment 5: Can you be more specific regarding the maternal/ uterine exclusion criteria eg using Thessaloniki ESHRE/ESGE consensus criteria. Similarly, are all women with fibroids &gt;5cm excluded, or only those with submucosal and/or intramural?

Response: Thanks for the comment. In this study, we will only focus on women with positive auto-antibody, and exclude women with other potential causes of abortion. We’ll use Thessaloniki ESHRE / ESGE consensus criteria, gynecological examination, ultrasound results and opinions of obstetricians and gynecologists, and exclude women with maternal anatomical abnormality. All women with fibroids &gt;5cm, no matter submucosal or intramucosal will be excluded.

Comment 6: ALT/ AST - again, are the thresholds for normality centrally defined or according the laboratory/ analyser? Are the reference ranges tailored for women of reproductive age?

Response: Thanks for the comments. The threshold for reference ranges of ALT/ AST will be defined according the laboratories in each hospital and apply to pregnant women. Given the potential hepatocellular and cholestatic injury of intrahepatic cholestasis of pregnancy (ICP) and the use of heparin, it’s necessary to monitor the level of ALT/ AST.

Comment 7: If you have to use sealed envelopes, can you include a method to verify that envelopes are used in order and not opened and resealed?

Response: Thanks for the comment. A special designed sealed opaque two-couplet random allocation envelope will be used for the randomization and allocation. We have added the details on the randomization section (page 9 line 208-213).
Comment 8: Are there any important prognostic variables you could use to stratify? With envelopes, it is probably only practical to stratify by one other variable, in addition to site.

Response: We don’t have any other variable for stratified randomization.

Comment 9: How is the dose of HCQ determined? Are the anticoagulants comparable in dose/efficacy? Who makes the decision as to which one?

Response: Thanks for the comments. The dose of HCQ is the maximal tolerance dose ranges from 100mg to 400mg daily, according to body weight. LMWH can be used with preventive dose and therapeutic dose. These three anticoagulants are all used with preventive dose in this study and are comparable in efficacy (Br J Haematol. 2003 Apr;121(1):12-20. DOI:10.1046/j.1365-2141.2003.04196.x ; Curr Med Res Opin. 2014 Mar;30(3):367-80. doi: 10.1185/03007995.2013.837818. Epub 2013 Nov 19. DOI:10.1185/03007995.2013.837818). Attending rheumatologists will decide which LMWH to use according to allergy history. We have revised the treatments section in the manuscript (page 10 line 215-219).

Comment 10: Is 24 weeks sufficiently long enough for pregnancy to occur? Other trials (eg ISRCTN92644181, ISRCTN15948785) use a window of 12 months. Are women who are using assisted conception eligible?

Response: We really appreciate this constructive comment. We completely agree with you that it’s a challenge for the women to conceive within 24 weeks. We extended 24 weeks to 12 months to ensure pregnancy to occur. Therefore, we have revised the manuscript (page 10 line 223, 230). Women who use assisted conception will be excluded for the purity of population.

Comment 11: Outcomes: What assessments are taking place every 4 weeks? How do these relate to the outcome measures.

Response: Thanks for the comments. The assessments include vital signs, lab test, side effects, current treatment record in order to detect adverse events on mother and fetus. The side effects are also one of the secondary outcomes.

Comment 12: Should the primary outcome be any live birth or term live birth? Premature delivery should include all live births before 34 and 37 weeks. Should you also include clinical pregnancy at 6-8 weeks and ongoing pregnancy at 12 weeks?

Response: Thanks for the helpful comment. The primary outcome is the live birth after at least 26 completed weeks of gestation. We only include women who try to conceive, and women who are already pregnant will be excluded. We have clarified in the outcome measures part (page 11 line 242).

Comment 13: Clearer definitions are required for maternal infection, gestational diabetes, UCTD activity, progression to CTD.
Response: We appreciate these helpful comments and have revised the manuscript as suggested. The outcome measures section now includes the further details of definitions on maternal infection, gestational diabetes, UCTD activity, progression to CTD (page 11 line 255-263).

Maternal infections include infections of upper and lower respiratory tract, gastrointestinal source, urinary tract and skin.

Gestational diabetes mellitus (GDM) is diabetes diagnosed in the second or third trimester of pregnancy based on “Standards of Medical Care in Diabetes” proposed by American Diabetes Association in 2015.

The purpose of assessing disease activity is to identify disease flare. Disease flare was defined with the presence of clinical manifestations such as arthritis, rash, Raynaud’s phenomenon, proteinuria with or without serology activity, which requires a new treatment or an increase of current dose or even hospitalization due to disease activity.

Progression to CTD is defined as evolution to SLE, APS, Sjogren’s syndrome, systemic sclerosis and other defined CTD diagnosed by classification criteria of American College of Rheumatology.

Comment 14: I calculate the sample size for the main comparison to be 121 women per group. Is the third group, HCQ and anticoagulant, are you recruiting the same number? You should adjust the sample size for multiple comparisons.

Response: We recalculated the sample size using PASS 15 for the main comparison to be 121 women per group and made corrections (page 12 line 279-283, page 2 line 40). Our primary objective is to compare hydroxychloroquine combined with low-dose prednisone and anticoagulation with anticoagulation alone in treating UCTD women with recurrent spontaneous abortion. The third arm of using hydroxychloroquine combined with anticoagulant for secondary comparison. Therefore, we did not conduct any multiple comparison adjustment in the sample size estimation. We have clarified this in the revised manuscript.

Comment 15: How confident are you that you will be able to obtain the primary outcome for all women? What proportion transfer between hospitals? How confident are you that 85% of eligible women will get pregnant within 24 weeks. For comparable UK trials, in ISRCTN92644181 53% of screened women were randomised (32% did not get pregnant) and in ISRCTN15948785, 42% did not get pregnant.

Response: We really appreciate your comments. There is a real challenge due to the uncertainty in the conception rate and live birth rate. Given the different pathophysiology of thyroid diseases and UCTD on pregnancy, UCTD is generally considered to be more associated with adverse pregnancy events rather than infertility. And we prolong the pre-conception phase to 1 year. Considering the family and social pressure during pregnancy in China, we expect the compliance rate is high. We will closely monitor our patients as well. In the setting of special social security for obstetric patients in China, most of the patients will labor in the same healthcare centers. We will also follow up with the patients who labor in the other medical centers.
Comment 16 : The known and expected adverse events of each drug should be stated (perhaps in an appendix) and those that are pertinent to miscarriage should be explicitly collected. Although all drugs and licenced and well used, but a process for identifying unexpected serious adverse drug reactions should be described.

Response: Thank you for the comment. Yes, we will collect the information on adverse events (see page 14 line 321-327 and Additional file 2). Rheumatologists are responsible for assessing the severity and the causality between the manifestations and the medications and adjusting further treatment plan accordingly.

Comment 17 : Analysis: You state the primary comparison is HCQ+prednisolone+anticoagulant versus anti-coagulant only. Are the other potential comparisons planned (HCQ+anticoagulant vs anticoagulant only, HCQ+prednisolone+anticoagulant vs HCQ+anticoagulant). If yes, the level of significance should be adjusted for multiple comparisons eg Bonferroni adjustment.

Response: Thank you for this excellent comment. No other comparison except for a secondary comparison between HCQ+prednisolone+anticoagulant and HCQ+anticoagulant. We have clarified this in the abstract and the main text (page2 line 36-40; page 9, line 201-204 and page 12, line 286-291).

Comment 18 : How will the Data Monitoring and Safety Board review data but keep the investigators blind to the accruing comparison data? On what grounds will they initiate the Safety Management Team?

Response: Thanks for your comments and the corresponding information has been edited in page 14 line 316-327. Members of DSMB need to sign a confidentiality agreement and fully protect patients’ privacy. Interim analyses of principal safety and effectiveness outcomes will be performed on behalf of DSMB by the trial statisticians who are blind to the treatment assignments. If serious adverse events occur, SMT will be initiated and may further make decision whether to remove the subject from the trial for safety reasons.

Comment 19 : What is the incidence rate of UCTD in women of reproductive age in China? Can the number of potentially eligible women seen in the hospitals be estimated? What proportion of this estimate will need to be randomised in the trial recruitment period?

Response: Thanks for your prudent comments. Spinillo et al. reported that UCTD was the most common rheumatic disease in pregnant women with a prevalence of 2.5% (62/2458) in Italy. Until now UCTD remains poorly studied that there is no exact incidence data of UCTD among women of reproductive age in China. Therefore, a current cross-section study regarding UCTD is conducted in our center and it is estimated that the incidence of UCTD among women of reproductive age is about 3%. It is estimated that around 250-300 women could be potentially eligible in our hospital per year.
Response to Reviewer 2: Prof. Zhengping Liu

The authors have planned a randomized trial to evaluate the efficacy of HCQ and low-dose prednisone on recurrent pregnancy loss for women with UCTD. This will be the first trial which evaluates the efficacy of immunosuppressant regimens on pregnancy outcomes and UCTD progression. If this trial completed, we can choose the best way to treat these patients. Therefore, we must be looking forward to the results of this trial. However, there still remain several concerns as follows.

Response: Thanks for the comments.

Comment 1 : Prednisone, HCQ, and other immune suppressant are commonly used for UCTD. In the anticoagulation alone group, the patients do not use immune suppressant? If their disease progresses, how to deal with them?

Response: Thank you for this excellent comment. Most patients with UCTD may remain stable for years with minimal disease activity. Moreover, it is not well established how or when pregnant patients with UCTD will progress to definite CTD. In the anticoagulation alone group, if the disease progressed, HCQ or prednisone will be used.

Comment 2 : There is no description about the blinding method. This is an open-label trial, however, the blinding method should be explained in the manuscript.

Response: Thank you for this prudent comment. This is an open-label trial and the description on blinding is added on page 9 line 204.

Comment 3 : This is a multi-center trial, and what I want to know is how did allocate the sample size in the six hospitals. What would the researchers do if the loss rate were different?

Response: Thanks for the comment. The sample size is allocated based on the size of the population of potentially eligible women in each center. Therefore, 226 patients will be enrolled in the main center, 40 patients in each of the other five hospitals. All physicians will be trained with regards to patient recruitment and follow-up, hopefully to minimize the missing data in the trial. We will consider appropriate statistical methods e.g. multiple imputation to impute missing data.

Comment 4 : It should be described whether a superiority analysis will be performed. The missing data handling method should also be described. Although a high rate of withdrawals is not expected, an adequate missing data treatment method should be put in place in advance in order to ensure the robustness and the credibility of the analysis. Finally, it should be stated whether a sensitivity analysis with the ‘per-protocol’ population is planned, along with other sensitivity analyses.

Response: We really appreciate this helpful comment. Further details have been added (page 9 line 201-204, page 12 line 286-292). This trial is to investigate the superior effect of HCQ+prednisolone+anticoagulant over anti-coagulant only in women with UCTD suffering
from RSA. We expect a high compliance rate for this patient population. We will also provide a
standardized training for all participating clinicians. In case there is missing data, we will assess
the nature of missingness and choose appropriate imputation methods e.g. multiple imputation.
All analyses will be by intention-to-treat supplemented by per-protocol analysis. A sensitivity
analysis will assess the robustness of the results to variation in the missing data assumptions

Minor point:

Comment 5: In the first part of background, "with a male-to-female ratio ranging from 1:5 to
1:17", please check the ratio again.

Response: Thank you for spotting this error. We have rechecked the ratio and made the
correction (page 4 line 73). Male-to-female ratio ranges from 1:15 to 1:17.

Comment 6: In the part recruitment, I do not understand the sentence "and the duration of
participation of each patient is from 24 weeks to 72 weeks".
Response: Thanks for your comments. We have revised the manuscript (page 7 line 161). The
duration of participation of each patient is estimated about 2 years, including the pre-conception
assessment around 1 year, pregnancy assessment of 42 weeks and post-partum assessment of 6
weeks.

Comment 7: The calculation of the sample size should be depicted in the abstract, as it is a
mainstay step in the design of the clinical trial.

Response: Thanks for the comment. Sample size has been added in the abstract on page 2 Line
38 and sample size calculation has been added in the sample size section on page 12 Line 281-
283.

Response to Reviewer 3: Prof. Amy Charlotte Brenner

The authors have submitted a protocol for a three-arm, open-label randomised controlled trial of
HCQ and prednisone (with LWMH alone as the control) for recurrent spontaneous abortion in
women with undifferentiated connective tissue disease. UCTD appears to be an important
problem in pregnancy and a trial in this area is reasonable given uncertainties surrounding patient
care and need for better treatments for pregnant women in general.

Response: Thanks for the comments.
BACKGROUND
The authors give a good rationale for the trial and provide adequate background information for a non-expert to grasp the public health problem and proposed trial.

Comment 1: Safety of mother and baby/fetus is always a concern when conducting trials in pregnant women. Have the authors reviewed the literature on safety of gestational use of the proposed interventions (HCQ and prednisone especially)? Several reviews have been conducted (e.g. https://www.ncbi.nlm.nih.gov/pubmed/21417950). It would be useful to summarise safety, particularly given that the authors are proposing treatment over the entire duration of pregnancy which will include several critical developmental stages in the fetus. The authors cite 3 trials of HCQ and prednisolone but only comment on pregnancy outcomes and not on adverse events. Presumably these trials were too small to detect AEs but it is still an important point that should be addressed. I note that the authors cite an observational study as evidence of a safety of HCQ use during pregnancy (Costedoat-Chalumeau et al. 2003) but the numbers of AEs are small and the groups were not randomised. I would like to know about the safety data reported in trials and the authors own pilot study...

Response: Thanks for the helpful comments. we did a review of literature and guidelines. Summary of this review has been added to the introduction section (page 5-6 line 108-136). ① In 2016 guideline on prescribing drugs in pregnancy and breastfeeding, corticosteroids and HCQ is compatible with each trimester of pregnancy, and HCQ should be continued during pregnancy in women planning a pregnancy with rheumatic disease in need of treatment. ② The therapeutic effect and side effect on pregnant women with UCTD are poorly studied and barely acknowledged due to small sample size. It has been well-established among patients with SLE or APS. And it is shown that there is no increased risk of congenital defects, spontaneous abortions, fetal death, prematurity or in patients with autoimmune diseases using HCQ. Therefore, HCQ is safe as the treatment for autoimmune diseases during pregnancy. ③ In our center, the results are promising regarding 66 UCTD patients with RSA treated by hydroxychloroquine plus low-dose prednisone combined with anticoagulation. There were 66 successful pregnancies (97.1% of live birth rate) and only 5 patients with obstetric complications in 68 pregnancies.

Comment 2: Page 5 line 111: The authors over-interpret the existing evidence on use of HCQ in CTD - authors should rephrase so as not to say 'has been proved to...', particularly since some of the cited studies are observational.

Response: We agree with you and have revised the manuscript accordingly (page 6 line 121-122).
METHODS

Comment 3 : It is unclear why the authors are conducting an open-label trial instead of blinding treatment allocation. Please can they justify this decision (why can't you use over-encapsulation and placebo pills to blind investigators and participants?). As long as allocation concealment is maintained and there is a good reason for using an open-label method this is acceptable, however.

Response: Thanks for the comment. We designed this trial as an open label trial due to the resource constraint. We have elaborated the allocation concealment in the manuscript (page 9, line 201-204)

Comment 4 : P7-8: Inclusion criteria are reasonable. Exclusion criteria are quite extensive and could limit generalisability and application of the trial findings. Any reason for excluding patients with history of malignancy, epilepsy or psychotic disorders?

Response: The exclusion criteria were defined based on the consideration that there are other causes of miscarriage. Prednisone and HCQ may affect the prognosis of malignancy, epilepsy or psychotic disorders, so we exclude patients with those above diseases.

Comment 5 : P8 line 182: How do the authors define ‘impossible to follow-up’?

Response: Thanks for comments. Even though we anticipate high compliance rate from this patient population, we will implement standard follow-up procedure. However, we might not be able to follow up patients whose residential addresses or contact information are changed.

Comment 6 : P9 line 192: Why does the dose of HCQ vary from 100mg to 200mg? And how were the dosage/regimen of the interventions determined?

Response: Thanks for the comments. The dose of HCQ is the maximal tolerance dose range from 100mg to 400mg daily, also according to body weight. We have clarified this in the manuscript on treatment section (page 10 line 216-220).

Comment 7 : P9 line 196: Why is treatment continued 6 weeks post-gestation if this study is aiming to treat RSA?

Response: Thanks for the comment. A disease flare or evolution can occur even in post-gestation stage and women in China usually back to the hospital for examination 6 weeks after delivery. So we suggest continuing to take oral medicine until the last visit at 6 weeks after delivery.

Comment 8 : P10 line 213: Is the primary outcome live births overall or among those who conceive? If the pre-conception interventions help with conception then but not RSA then you will be mixing the two effects if measuring live births overall (those in the treatment group may have a greater number of women who conceive but a similar number of RSA's, for example). Please clarify.
Response: We really appreciate your constructive comment. The primary outcome is live births among those pregnant women. However, we will also take into account the conception rate as one of the secondary outcomes.

Comment 9 : P10 line 216: Secondary outcomes should include stillbirth rate as well as miscarriage. Consider rate of conception too? What about other maternal morbidities such as obstetric bleeding (antepartum haemorrhage, postpartum haemorrhage), and placental abnormalities eg placenta praevia.

Response: Thanks for the helpful comment. Corresponding content has been added to the manuscript (page 11 line 245-254). Stillbirth rate and miscarriage are both calculated among pregnant patients. Other maternal morbidities will be considered as secondary outcome measures as well.

Comment 10 : P11 line 247: It is not clear how reference 43 provides evidence on the likely rate of loss to FU due to women not conceiving during the 24 week conception period. Please clarify. I think it may be ambitious to expect 85% of women to conceive within 6 weeks... I suggest a larger sample size, particularly given the uncertainty surrounding the event rate in the control group and treatment effect size. A trial with 80% power still has a relatively large (20%) chance of missing a treatment effect if one truly exists. The authors should round up their estimated sample size since the calculation is based on several assumptions. I cannot match the calculation exactly - the authors should specify what relative increase in live births they based the calculation on e.g. a risk ratio of 1.20... The authors also need to consider the impact on non-adherence to treatment and loss to follow-up in terms of missing outcome data. If the authors truly want to conduct a definitive, large scale study, then I suggest increasing the sample size.

Response: We really appreciate your comments and revised the manuscript (page 12 line 282-284). Per reference 44 instead of 43, 65.7% became pregnant within 6 months. We have to admit it as a challenge to set 24 weeks as conception period. Therefore, we prolong the pre-conception phase to 1 year (page 10 line 230-231), recalculate the sample size to 426 (142 in each group). Considering the family and social pressure during pregnancy in China, most patients are compliant to treatment and follow-up. We will closely monitor our patients as well.

Comment 11 : The authors should mention their dissemination strategy and data sharing plans.

Response: We really appreciate your precise comment. We have added the corresponding content to the dissemination section (page 14 line 325-327).

Response to Reviewer 4: Prof. Zainab Alimoradi

Thanks for inviting me to review this manuscript. This is a well written protocol for a randomized controlled trial. All details of study is explained. Some minor point needs to be considered.

Response: Thanks for the comments.
Comment 1: In line 109-110: it is better to explain the limitation of previous studies which leads to importance of present study. If low sample size is the only point, it is better for reader to have some information about that.

Response: Thank you for this excellent comment, Prof. Zainab Alimoradi. We really appreciate it. We have revised the manuscript (page 5-6 line 113-117, page 15 line 345-347). UCTD was the most common rheumatic disease in pregnant women. But the effects of UCTD treatments on pregnant outcomes is poorly studied and barely acknowledged. Most studies are observational studies with small scale instead of randomized controlled study. We take references from the treatment of recurrent miscarriage, SLE and APS. It’s necessary to conduct a large scale, randomized controlled trial to investigate the therapeutic effect and side effect of medications on the pregnant women suffering from UCTD.

Comment 2: Line 118-121: please provide citation.

Response: Thanks for the comments. It’s the unpublished cohort data from our hospital.

Comment 3: In randomization section, why stratification is implemented by sites?

Response: Thanks for the comments. The stratification randomization by site is meant to offer the best balance in the number of patients between groups among the study sites.

Comment 4: In calculating sample size, it is better to consider MCID approach too.

Response: Thanks for the helpful comment and we totally agree with you. MCID approach is of good prospects in application. In this trial, based on limited published data, it is hypothesized that the proportion of live birth rates with immunosuppressant and anticoagulation treatment is 85% vs 70% for anticoagulant only. We need to enroll 363 women (121 in each group) to allow us to detect the difference with a power of 0.8 at a two-sided P value of 0.05. We planned to enroll 426 participants (142 in each group) considering unpregnant rate and loss to follow-up.