PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Comparison of PGS2.0 versus conventional embryo morphology evaluation for patients with recurrent pregnancy loss: a study protocol for a multicentre randomised trial
AUTHORS	Lei, Caixia; Sui, Yilun; Ye, Jiangfeng; Lu, Yao; Xi, Ji; Sun, Yun; Jin, Li; SUN, XIAOXI

VERSION 1 – REVIEW

REVIEWER	Vu N. A. Ho IVFMD, My Duc Hospital, HCMC, Viet Nam
REVIEW RETURNED	29-Dec-2019

GENERAL COMMENTS	Review report Comparison of PGS 2.0 versus conventional embryo morphology evaluation for patients with recurrent pregnancy loss: a study protocol for a multicentre prospective randomised trial • Summary of the study This is a protocol for an RCT comparing the effectiveness between PGS 2.0 and conventional embryo morphology evaluation in patients with recurrent pregnancy loss Hence, term "PGS" should be replaced to "PGT" throughout the protocol. • Overall opinion on the manuscript and recommendations The topic is new and interesting. There are several issues which should be concerned by authors. However, I suggest this manuscript to be accepted with minor revison. • General comments 1. There is no need of “prospective” before “randomised trial”. 2. Currently, the term “PGS” has not been used anymore; so, authors should add information of the new term “PGT-A” from the beginning in Abstract. 3. Could the author explain the role of “testosterone” in basal hormonal check-up? 4. For the usage of GnRH antagonist, which recommendation do authors follow? 5. Is that correct that 100% of patients having hCG trigger while there may be patients with hyper-response in this study? Although the freeze-only strategy is used, does the author concern about the potential risk of ovarian hyper-stimulation syndrome on those patients? 6. A 30-minute bed rest should not be recommended rountinely as immediate mobilization after an embryo transfer does not have a negative influence over the success rates of IVF. Ref: Cozzolino M, Troiano G, Esencan E. Bed rest after an embryo transfer: a systematic review and meta-analysis. Arch Gynecol Obstet. 2019 Nov;300(5):1121-1130. 7. The definition of live birth rate that the authors are using based on which recommendation?

	8. The term “miscarriage” is used to define spontaneous loss of a clinical pregnancy before 22 completed weeks of gestational age, in which the embryo(s) or fetus(es) is/are nonviable and is/are not spontaneously absorbed or expelled from the uterus. The outcome “miscarriage” definition that authors mention should be checked again. Ref: Zegers-Hochschild F, Adamson GD, Dyer S, et al. The International Glossary on Infertility and Fertility Care, 2017. Hum Reprod. 2017;32(9):1786–1801. 9. Information on Clinical trials registration should be mentioned in detailed in the protocol. • Minor changes 1. Authors should check the in-text citation again; for example: “abnormalities,[4]” should be corrected as “abnormalities4, etc. 2. Page 4, line 10, “procedures are performed” should be “procedure is performed” 3. Page 7, line 14, it should be “chromosomal status21, given…” 4. Page 8, line 38, “consents” not “consent” 5. Page 9, line 4, it should be “3. Female aged between 20 and 38 years.” 6. Page 9, line 48, “once a good quality embryo” should be clarified as “once a good quality embryo or a normal embryo after PGT-A” 7. Page 15, line 59, “Sample size calculation” should be in bold.

REVIEWER	Federico Cirillo Humanitas Clinical and Research Center, IRCCS Department of Gynecology, Division of Gynecology and Reproductive Medicine Humanitas Fertility Center via Manzoni 56, 20089 Rozzano (Milan) Italy tel. +390282244646
REVIEW RETURNED	23-Mar-2020

GENERAL COMMENTS	Study population/participants and recruitment Page 10, line 54-55: 3 PLs is a more common definition in the clinical practice. Page 11, line 4-5: for the inclusion criteria, is there any criteria about ovarian reserve? is there any criteria about infertility or medical history? Are there any limitations/investigations (apart from the already mentioned karyotype) regarding the male partner? Page 11, line 9-10: for uterine abnormalities, will all the patients be screened with hysteroscopy or 3D US? Page 11, line 22-23: will all the patients be screened for thrombosis? Randomisation Page 12, line 6-7: at which time will the randomization take place (pick up, first day of stimulation protocol...)? Please provide more info about the randomization technique, it’s not very clear. Questionnaire Page 10, line 11-15: Why no male information is collected? COH protocol Page 12, line 37-38: this part in not clear and it is in complete discordance with the previous M&M section, or not clear. From the first part (line 30) it seemed that patients are enrolled for single COH cycle. Explain better in detail

	Page 13, line 59-60: provide a reference for these kind of COH, because they are not worldwide uniform. How do you decide the starting dose? Page 14, line 9-10: no trigger with agonist? Page 14 line 22: its not clear according to your centre when you decide to stop a cycle and when and how you change protocol Page 14 line 25: before line 25 is not that clear you want to use just ICSI as procedure, underline it better. The only other reference point when you talk about ICSI is at line 33 of page 10. It is interesting as concept but underline it more and explain why you use just ICSI as procedure. Good quality embryo evaluation Page 15, line 6-7: How does your centre specifically assesses good morphology? Page 16, line 1-17: Again, here better specify what happens if the patients are not pregnant Page 17, line 40-48: not very clear, maybe rephrase. Overall: very interesting study of very important clinical relevance, considering the great debate on the topic at the moment. Clear, well-structured and well written with a good use of the English vocabulary. Good knowledge of the literature is seen throughout the paper.

REVIEWER	Andreas Schmutzler Kiel University, Germany
REVIEW RETURNED	23-Mar-2020

GENERAL COMMENTS	The authors describe the protocol of a running multicenter RCT, the first of its kind, with an intended amount of 268 couples with recurrent pregnancy loss (RPL), in order to investigate the effect of PGT-A vs. morphology only, in single blastocyst frozen transfers. Form - Appropriate in all parts, 51 % of the literature is from the last 5 years. Content Abstract - It must read that sample size calculations are based on a difference of 15%-POINTS, not “on a 15% difference”. Introduction - The definition of RPL of two or more PL is not globally accepted, more reference must be given. - Pure blastocyst cultivation is not globally considered to have no negative effects, more reference must be given. - The authors cite an unpublished analysis of data with LBR per initiated cycle of 27% in RPL with PGS and 15% without, i. e. a difference of 12%-points. This is why their aim of 15%-points is two ambitious, mostly due to a low number of included patients, and most probably will not show a difference between the two groups, which will result in another failed RCT in this field. An increase of 5%- to 10%-points clinically would be relevant but not detected. And the opponents of PGS will claim again that it is again proven that this method is useless. From this point of view, one could argue that this study might not only be useless but also dangerous and probably counterproductive! The ESTEEM and STAR trials should be discussed in relation to the trial intended here. Methods

	- No blinding: it should be discussed that there is a danger that the PGS group might get a higher stimulation in order to get more oocytes for selection. - All patients get blastocyst cultivation: As LBR per initiated cycle it the primary study aim, and not the reduction of miscarriages, this makes no sense when there is only one fertilized oocyte or only one eight cell embryo on day three, as then a selection is not possible and chances of pregnancy might get reduced by a longer cultivation. This might reduce the chances in both groups, but especially in the PGS group, as these embryos additionally get a biopsy. - All blastocysts will be frozen: it should be discussed with references that it is not globally accepted that this does no harm to both groups, especially as in the control group there is normally no compulsory reason to do so. - Sample size calculation: it reads minimum “will be 242 participants for each group” – this seems to be wrong. Sample size calculation - The three participating centers give an LBR of 15% without and 30% with PGS within the last three years. So, they try to prove a doubling of their results whereas an increase of one third, i.e. by 33% or 5%-points, from 15% to 20%, clinically would be already very relevant. Normally this very high aim is chosen because of economics, as to detect 5%-points more is much more expensive and time consuming, as it requires much more patients. A cheaper negative finding gets published as well, only for the knowledge about the real clinical value of PGS that contribution might be very limited. Ethics - It is said that PGS might decrease LBR in older patients but here this would be the first study in younger ones: the inclusion of 20 to 38 years does not make it a young group but includes also advanced maternal age (AMA). - It is said that “this study may prove that PGS is a quick and safe future treatment option.”: additional burdens of time, “nerves” and especially money should be mentioned. SPIRIT checklist - 39% of the questions are answered by N/A. This is not correct and should be optimized. In sum: This RCT of cause should be published, but its danger to further compromise the advantages of PGS without being able to find clinically very relevant information about it, should be mentioned from the very beginning!

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Vu N. A. Ho

Institution and Country: IVFMD, My Duc Hospital, HCMC, Viet Nam

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

Please leave your comments for the authors below

Review report

Comparison of PGS 2.0 versus conventional embryo morphology evaluation for patients with recurrent pregnancy loss: a study protocol for a multicentre prospective randomised trial

• Summary of the study

This is a protocol for an RCT comparing the effectiveness between PGS 2.0 and conventional embryo morphology evaluation in patients with recurrent pregnancy loss Hence, term "PGS" should be replaced to "PGT" throughout the protocol.

• Overall opinion on the manuscript and recommendations

The topic is new and interesting. There are several issues which should be concerned by authors. However, I suggest this manuscript to be accepted with minor revison.

• General comments

1. There is no need of “prospective” before “randomised trial”.
   Thank you for your advice, “prospective” is deleted in the maintext.

2. Currently, the term “PGS” has not been used anymore; so, authors should add information of the new term “PGT-A” from the beginning in Abstract.
   Thank you for your advice. PGT is now used and accepted worldwide and we are using the term now, however, we here use PGS2.0 to distinguish with PGS1.0 which is based mostly on FISH technology. PGS2.0 here is properly though PGS is an old-fashioned term.

3. Could the author explain the role of “testosterone” in basal hormonal check-up?
   This is a regular test in our clinic to check if some of the patients had hyperandrogenemia and we can treat these patients before the start of the stimulation.

4. For the usage of GnRH antagonist, which recommendation do authors follow?
   We have applied fixed antagonist protocol with Cetrotide applicated on the 7th day of Gonodotropin (rFSH or HMG). However, the ovarian response will be checked on the 6th day of Gn, if we find that E2 level is above 1000 pg/ml or follicle more than 14mm or LH > 15 IU/L, we will add Cetrotide to adjust to flexible scheme.
   Ref: Bar Hava I, Blueshtein M, Ganer Herman H, Omer Y, Ben David G. Gonadotropin-releasing hormone analogue as sole luteal support in antagonist-based assisted reproductive technology cycles. Fertility and sterility 2017; 107 (1): 130-135 e131. doi: 10.1016/j.fertnstert.2016.10.011.

5. Is that correct that 100% of patients having hCG trigger while there may be patients with hyper-response in this study? Although the freeze-only strategy is used, does the author concern about the potential risk of ovarian hyper-stimulation syndrome on those patients?
   We have designed this RCT to all use hCG trigger at first, but now some of the patients truly use the agonist trigger, so we have revised in the main text, thank you for your advice.
   It is correct that although we use freeze-all strategy, the potential risk of ovarian hyper-stimulation syndrome could happen on some of these patients. We will record these adverse events and give appropriate and timely treatment.

6. A 30-minute bed rest should not be recommended rountinely as immediate mobilization after an embryo transfer does not have a negative influence over the success rates of IVF.

Ref: Cozzolino M, Troiano G, Esencan E. Bed rest after an embryo transfer: a systematic review and meta-analysis. Arch Gynecol Obstet. 2019 Nov;300(5):1121-1130.

Thank you for your suggestion, we all agree your opinion. We have revised in the manuscript. Page 13 "The patients will lie in bed for half an hour or be free to walk around after transfer.".

6. The definition of live birth rate that the authors are using based on which recommendation?
Live birth will be defined as a live born baby with a gestational period beyond gestational week 28, and birth weight more than 1000 g according to the Chinese guidelines.

Ref: Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, et al. Transfer of Fresh versus Frozen Embryos in Ovulatory Women. The New England journal of medicine 2018; 378 (2): 126-136. doi: 10.1056/NEJMoa1705334.

8. The term "miscarriage" is used to define spontaneous loss of a clinical pregnancy before 22 completed weeks of gestational age, in which the embryo(s) or fetus(es) is/are nonviable and is/are not spontaneously absorbed or expelled from the uterus.

The outcome "miscarriage" definition that authors mention should be checked again.

Ref: Zegers-Hochschild F, Adamson GD, Dyer S, et al. The International Glossary on Infertility and Fertility Care, 2017. Hum Reprod. 2017;32(9):1786–1801.

We agree the term, however according to the Chinese guidelines, miscarriage will be defined as the termination of the pregnancy at <28 weeks of gestation with a miscarried foetal weight less than 1000 g. But mostly the miscarriage happens before 12 weeks of gestation.

Ref: Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, et al. Transfer of Fresh versus Frozen Embryos in Ovulatory Women. The New England journal of medicine 2018; 378 (2): 126-136. doi: 10.1056/NEJMoa1705334.

Recurrent pregnancy loss is controversial on how many times of losses, two or three, should be considered to have treatment. Now many guidelines and committee opinion recommend if patients have 2 PLs should be properly treated to lower the risk of PL.

Ref: Group EEPGD. RECURRENT PREGNANCY LOSS Guideline of the European Society of Human Reproduction and Embryology. 2017. www.eshre.eu/guidelines, Last update: NOVEMBER 2017.

Ref: Practice Committee of American Society for Reproductive M. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertility and sterility 2013; 99 (1): 63. doi: 10.1016/j.fertnstert.2012.09.023.

9. Information on Clinical trials registration should be mentioned in detailed in the protocol.

Thank you for your advice, we have revised in the main text on page 18: "The study was designed in July 2017, and the first participant was randomised on March 22, 2018. At the time of the manuscript preparation, we have recruited 100 couples and the recruitment is ongoing. Trial registration number: NCT03214185 and stage: Pre-results. We aim to complete the recruitment by March 31, 2021.".

• Minor changes

1. Authors should check the in-text citation again; for example: “abnormalities,[4]” should be corrected as “abnormalities4, etc.
   Thank you, I have followed the editor’s advice and changed to this style.

2. Page 4, line 10, “procedures are performed” should be “procedure is performed”
   Thank you for your advice, we have revised in the main text.

3. Page 7, line 14, it should be “chromosomal status21, given…”
   Thank you, I have followed the editor’s advice and changed to this style.

4. Page 8, line 38, “consents” not “consent”
   Thank you for you kindly check, I have corrected it.

5. Page 9, line 4, it should be “3. Female aged between 20 and 38 years.”
   Thank you for you kindly check, I have corrected it.

6. Page 9, line 48, “once a good quality embryo” should be clarified as “once a good quality embryo or a normal embryo after PGT-A”
   Thank you for you kindly check, I have corrected it.

7. Page 15, line 59, “Sample size calculation” should be in bold.
   Thank you for you kindly check, I have corrected it.

Reviewer: 2

Reviewer Name: Federico Cirillo

Institution and Country: Humanitas Clinical and Research Center, IRCCS

Department of Gynecology, Division of Gynecology and Reproductive Medicine

Humanitas Fertility Center

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

Please leave your comments for the authors below

Study population/participants and recruitment

Page 10, line 54-55: 3 PLs is a more common definition in the clinical practice.

Now many guidelines and committee opinion recommend if patients have 2 PLs should be properly treated to lower the risk of PL.

Ref: Group EEPGD. RECURRENT PREGNANCY LOSS Guideline of the European Society of Human Reproduction and Embryology. 2017. www.eshre.eu/guidelines, Last update: NOVEMBER 2017.

Ref: Practice Committee of American Society for Reproductive M. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertility and sterility 2013; 99 (1): 63. doi: 10.1016/j.fertnstert.2012.09.023.

Page 11, line 4-5: for the inclusion criteria, is there any criteria about ovarian reserve? is there any criteria about infertility or medical history? Are there any limitations/investigations (apart from the already mentioned karyotype) regarding the male partner?

The aim of the study is to focus on clinical outcome of RPL couples using PGT-A. For the inclusion criteria, there is no criteria about ovarian reserve for we all know poor ovarian response will reduce the number of retrieved oocytes, viable day three embryos and blastocysts, but the euploid embryos may also be reduced. We could adjust these confounders with statistical methods when analyze the clinical outcome if these patients are included.

Though we do not mention about infertility, the included patients go to IVF clinic to seek help not just about RPL, but about infertility which might be caused by frequent uterine cavity operation after abortion.

There are some investigations regarding the male partner, the analysis of Semen examination will be collected during the trial.

Page 11, line 9-10: for uterine abnormalities, will all the patients be screened with hysteroscopy or 3D US?

All the patients will be screened with 2D US for uterine abnormalities.

Page 11, line 22-23: will all the patients be screened for thrombosis?

All the patients will be screened for thrombosis.

Randomisation

Page 12, line 6-7: at which time will the randomization take place (pick up, first day of stimulation protocol...)? Please provide more info about the randomization technique, it's not very clear.

Randomization will be taken place at the day of the first visit of the couples to the clinic or on the first day of stimulation. We have added this to the revised version, thank you for your kindly suggestion.

Questionnaire

Page 10, line 11-15: Why no male information is collected?

Male information is collected in this questionnaire; the questionnaire is designed for female partner to fulfill however information of both of the male and female will be collected together.

COH protocol

Page 12, line 37-38: this part in not clear and it is in complete discordance with the previous M&M section, or not clear. From the first part (line 30) it seemed that patients are enrolled for single COH cycle. Explain better in detail

Thank you for your advice. All patients will undergo three COH cycles unless they become pregnant after the first or second cycle, or they indicate that they wish to stop treatment. If the patient is not pregnant after three COH cycles, she will be automatically withdrawn from the study. If she has surplus embryos, she could continue to have transfer cycles in the following menstrual period.

Page 13, line 59-60: provide a reference for these kind of COH, because they are not worldwide uniform. How do you decide the starting dose?

Ref: Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam WS, Broekmans FJ. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. The Cochrane database of systematic reviews 2016; 4 CD001750. doi: 10.1002/14651858.CD001750.pub4.

The initiative doses will be 150–300 IU/day according to female age, body mass index (BMI), number of antral follicles, and basal hormone levels.

Page 14, line 9-10: no trigger with agonist?

We have designed this RCT to all use hCG trigger at first, but now some of the patients truly use the agonist trigger, so we have revised in the main text, thank you for your advice.

Page 14 line 22: its not clear according to your centre when you decide to stop a cycle and when and how you change protocol

If some of the patients decide to stop a cycle for personal reasons, we will stop the treatment. We do not change the antagonist protocol unless the patients have no response.

Page 14 line 25: before line 25 is not that clear you want to use just ICSI as procedure, underline it better. The only other reference point when you talk about ICSI is at line 33 of page 10. It is interesting as concept but underline it more and explain why you use just ICSI as procedure.

We choose ICSI for the purpose of reduce the spermatozoa contamination during biopsy. And for the control group, ICSI is chosen to be consistent with the PGS group to reduce bias.

Good quality embryo evaluation

Page 15, line 6-7: How does your centre specifically assesses good morphology?

We have added the reference of our center to assess the morphology of the embryos. Evaluation of blastocyst stage embryos are based on three aspects: the expansion of the blastocoele cavity (EH stage), the number and cohesiveness of the inner cell mass (ICM grade) and trophectodermal cells (TE grade) according to the Gardner and Schoolcraft grading system34-36. The EH stage is assessed as one of the following: (1) an early blastocyst, blastocoele being less than half volume of that of the embryo; (2) a blastocyst with a blastocoele whose volume is at least half that of the embryo; (3) a full blastocyst with a blastocoele completely filling the embryo; (4) an expanded blastocyst with a blastocoele volume larger than that of the full blastocyst, with a thinning zona; (5) a hatching blastocyst with the TE starting to herniate through the zona; and (6) a hatched blastocyst, in which the blastocyst has completely escaped from the zona. ICM and TE grade are evaluated after EH stage is assessed. The ICM is assessed as one of the following: (A) tightly packed, many cells; (B) loosely grouped, several cells; and (C) very few cells. The TE is assessed as one of the following: (A) many cells forming a cohesive epithelium; (B) few cells forming a loose epithelium; and (C) very few, large cells.

Ref: Gu R, Feng Y, Guo S, Zhao S, Lu X, Fu J, et al. Improved cryotolerance and developmental competence of human oocytes matured in vitro by transient hydrostatic pressure treatment prior to vitrification. Cryobiology 2017; 75 144-150. doi: 10.1016/j.cryobiol.2016.12.009.

Fu J, Shao J, Li X, Xu Y, Liu S, Sun X. Non-invasive metabolomic profiling of day 3 embryo culture media using near-infrared spectroscopy to assess the development potential of embryos. Acta Biochim Biophys Sin (Shanghai) 2013; 45 (12): 1074-1078. doi: 10.1093/abbs/gmt115.

Page 16, line 1-17: Again, here better specify what happens if the patients are not pregnant.

If the patient is not pregnant after three COH cycles, she will be automatically withdrawn from the study. If she has surplus embryos, she could continue to have transfer cycles in following menstrual period.

Page 17, line 40-48: not very clear, maybe rephrase.

Thank you for your advice. We have modified in the main text. "The study was designed in July 2017, and the first participant was randomised on March 22, 2018. At the time of the manuscript preparation, we have recruited 100 couples and the recruitment is ongoing. Trial registration number: NCT03214185 and stage: Pre-results. We aim to complete the recruitment by March 31, 2021."

Overall: very interesting study of very important clinical relevance, considering the great debate on the topic at the moment. Clear, well-structured and well written with a good use of the English vocabulary. Good knowledge of the literature is seen throughout the paper.

Reviewer: 3

Reviewer Name: Andreas Schmutzler

Institution and Country: Kiel University, Germany

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

The authors describe the protocol of a running multicenter RCT, the first of its kind, with an intended amount of 268 couples with recurrent pregnancy loss (RPL), in order to investigate the effect of PGT-A vs. morphology only, in single blastocyst frozen transfers.

Form

- Appropriate in all parts, 51 % of the literature is from the last 5 years.

Content

Abstract

- It must read that sample size calculations are based on a difference of 15%-POINTS, not "on a 15% difference".

Thank you for your advice. We have corrected in the main text. "Sample size calculation will be based on a difference of 15%-points in the LBR per initiated cycle between the two cohorts, and a smaller difference in the LBR may not be detected."

Introduction

- The definition of RPL of two or more PL is not globally accepted, more reference must be given.

Thank you for your advice. We have corrected in the main text. Recurrent pregnancy loss is controversial on how many times of losses, two or three, should be considered to have treatment. Now many guidelines and committee opinion recommend if patients have 2 PLs should be properly treated to lower the risk of PL.

Ref: Group EEPGD. RECURRENT PREGNANCY LOSS Guideline of the European Society of Human Reproduction and Embryology. 2017. www.eshre.eu/guidelines, Last update: NOVEMBER 2017.

Ref: Practice Committee of American Society for Reproductive M. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertility and sterility 2013; 99 (1): 63. doi: 10.1016/j.fertnstert.2012.09.023.

- Pure blastocyst cultivation is not globally considered to have no negative effects, more reference must be given.

Thank you for your advice. We choose blastocyst cultivation because PGS2.0 tend to trophectoderm biopsy and elective single blastocyst transfer, and the clinical pregnancy rate of blastocyst transfer is higher than cleavage-stage embryo transfer.

Ref: Reljic M, Knez J, Kovac V, Kovacic B. Endometrial injury, the quality of embryos, and blastocyst transfer are the most important prognostic factors for in vitro fertilization success after previous repeated unsuccessful attempts. Journal of assisted reproduction and genetics 2017; 34 (6): 775-779. doi: 10.1007/s10815-017-0916-4.

- The authors cite an unpublished analysis of data with LBR per initiated cycle of 27% in RPL with PGS and 15% without, i. e. a difference of 12%-points. This is why their aim of 15%-points is two ambitious, mostly due to a low number of included patients, and most probably will not show a difference between the two groups, which will result in another failed RCT in this field. An increase of 5%- to 10%-points clinically would be relevant but not detected. And the opponents of PGS will claim again that it is again proven that this method is useless. From this point of view, one could argue that this study might not only be useless but also dangerous and probably counterproductive! The ESTEEM and STAR trials should be discussed in relation to the trial intended here.

We have published the retrospective data in 2019, and based on cost-benefit accounting, the sample size based on the 15% increase is appropriate.

Ref: Lei C-X, Ye J-F, Sui Y-L, Zhang Y-P, Sun X-X. Retrospective cohort study of preimplantation genetic testing for aneuploidy with comprehensive chromosome screening versus nonpreimplantation genetic testing in normal karyotype, secondary infertility patients with recurrent pregnancy loss. Reproductive and Developmental Medicine 2019; 3 (4): 205-212. doi: 10.4103/2096-2924.274544.

Methods

- No blinding: it should be discussed that there is a danger that the PGS group might get a higher stimulation in order to get more oocytes for selection.

The dose of the Gonadotropins and euploidy rate is controversial. We use the randomized trial to reduce confounders.

The initiative doses will be 150–300 IU/day according to female age, body mass index (BMI), number of antral follicles, and basal hormone levels. To choose PGS or not is not considered when choose the initiative stimulation dose, and the adjustment of dose will be based on the women's ovarian response.

Ref: McCulloh DH, Alikani M, Norian J, Kolb B, Arbones JM, Munne S. Controlled ovarian hyperstimulation (COH) parameters associated with euploidy rates in donor oocytes. Eur J Med Genet 2019; 62 (8): 103707. doi: 10.1016/j.ejmg.2019.103707.

Wu Q, Li H, Zhu Y, Jiang W, Lu J, Wei D, et al. Dosage of exogenous gonadotropins is not associated with blastocyst aneuploidy or live-birth rates in PGS cycles in Chinese women. Human reproduction 2018; 33 (10): 1875-1882. doi: 10.1093/humrep/dey270.

- All patients get blastocyst cultivation: As LBR per initiated cycle it the primary study aim, and not the reduction of miscarriages, this makes no sense when there is only one fertilized oocyte or only one eight cell embryo on day three, as then a selection is not possible and chances of pregnancy might get reduced by a longer cultivation. This might reduce the chances in both groups, but especially in the PGS group, as these embryos additionally get a biopsy.

It's controversial if PGT-A should be applied to poor response patients or advanced maternal age patients for their poor clinical outcome because of the least number of viable embryos. However, the transfer of embryos without PGT-A might cause higher risk of pregnancy loss in this particular population. Abandoning PGT-A due to poor response to ovarian stimulation is not a favorable option.

Ref: Sacchi L, Albani E, Cesana A, Smeraldi A, Parini V, Fabiani M, et al. Preimplantation Genetic Testing for Aneuploidy Improves Clinical, Gestational, and Neonatal Outcomes in Advanced Maternal Age Patients Without Compromising Cumulative Live-Birth Rate. Journal of assisted reproduction and genetics 2019; 36 (12): 2493-2504. doi: 10.1007/s10815-019-01609-4.

- All blastocysts will be frozen: it should be discussed with references that it is not globally accepted that this does no harm to both groups, especially as in the control group there is normally no compulsory reason to do so.

It is correct that although we use freeze-all strategy, the potential risk of ovarian hyper-stimulation syndrome could happen on some of these patients. We will record these adverse events and give appropriate and timely treatment.

- Sample size calculation: it reads minimum "will be 242 participants for each group" – this seems to be wrong.

Thank you for your advice, we have corrected it in the main text. "The number will be set to 1:1 in each group, and the minimum sample size will be 242 participants."

Sample size calculation

- The three participating centers give an LBR of 15% without and 30% with PGS within the last three years. So, they try to prove a doubling of their results whereas an increase of one third, i.e. by 33% or 5%-points, from 15% to 20%, clinically would be already very relevant. Normally this very high aim is chosen because of economics, as to detect 5%-points more is much more expensive and time consuming, as it requires much more patients. A cheaper negative finding gets published as well, only for the knowledge about the real clinical value of PGS that contribution might be very limited.

We have published the retrospective data in 2019, and based on cost-benefit accounting, the sample size based on the 15% increase is appropriate.

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Ethics

- It is said that PGS might decrease LBR in older patients but here this would be the first study in younger ones: the inclusion of 20 to 38 years does not make it a young group but includes also advanced maternal age (AMA).

It's controversial if PGT-A should be applied to poor response patients or advanced maternal age patients for their poor clinical outcome because of the least number of viable embryos. However, the transfer of embryos without PGT-A might cause higher risk of pregnancy loss in this particular population. Abandoning PGT-A due to poor response to ovarian stimulation is not a favorable option.

Ref: Sacchi L, Albani E, Cesana A, Smeraldi A, Parini V, Fabiani M, et al. Preimplantation Genetic Testing for Aneuploidy Improves Clinical, Gestational, and Neonatal Outcomes in Advanced Maternal Age Patients Without Compromising Cumulative Live-Birth Rate. Journal of assisted reproduction and genetics 2019; 36 (12): 2493-2504. doi: 10.1007/s10815-019-01609-4.

We choose to include women under 38 years according to the committee opinion and Chinese guideline toward PGT-A.

Ref: Practice Committees of the American Society for Reproductive M, the Society for Assisted Reproductive Technology. Electronic address Aao, Practice Committees of the American Society for Reproductive M, the Society for Assisted Reproductive T. The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. Fertility and sterility 2018; 109 (3): 429-436. doi: 10.1016/j.fertnstert.2018.01.002.

HF H, J Q, JY L, ZJ C, YX C, LQ W, et al. Consensus on Preimplantation Genetic Dignosis/ Screening. Chia J Med Genec 2018; 35 (2): 151-155. doi: 10.3760/cma.j.issn.1003-9406.2018.02.001.

- It is said that "this study may prove that PGS is a quick and safe future treatment option.": additional burdens of time, "nerves" and especially money should be mentioned.

It's true that for young infertile couples without medical history of recurrent pregnancy loss, PGS might be time consuming and costive, however, for RPL couples, it might be a quick and safe treatment for the burdens of time and cost during miscarriage is reduced.

Ref: Garcia-Velasco JA, Fauser BC. Preimplantation genetic screening - what a wonderful world it would be! Reproductive biomedicine online 2016; 32 (4): 337-338. doi: 10.1016/j.rbmo.2016.02.007.

SPIRIT checklist

- 39% of the questions are answered by N/A. This is not correct and should be optimized.

Thank you for your advice, we have corrected it in the main text and fulfilled SPIRIT checklist.

In sum: This RCT of cause should be published, but its danger to further compromise the advantages of PGS without being able to find clinically very relevant information about it, should be mentioned from the very beginning!

VERSION 2 – REVIEW

REVIEWER	Federico Cirillo IRCCS Istituto Clinico Humanitas Fertility Center Rozzano (MI), Italy
REVIEW RETURNED	27-Jun-2020

GENERAL COMMENTS	I appreciate the effort that the authors spent on the revision and I would suggest the paper for pubblication.

REVIEWER	PD DR. Andreas Schmutzler University Women's Hospital, Kiel, Germany
REVIEW RETURNED	03-Jul-2020

GENERAL COMMENTS	Good luck with your study!