PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Pre-IVF treatment with a GnRH antagonist in women with endometriosis (PREGnant): Study protocol for a prospective, double-blind, placebo-controlled trial
AUTHORS	Taylor, Hugh; Li, Howard; Carson, Sandra; Flores, Valerie; Pal, Lubna; Robbins, Jared; Santoro, Nanette F.; Segars, James H.; Seifer, David; Huang, H; Young, Steven; Zhang, Heping

VERSION 1 – REVIEW

REVIEWER	Laganà, Antonio Simone University of Insubria
REVIEW RETURNED	11-Jun-2021

GENERAL COMMENTS	I read with great interest the manuscript, which falls within the aim of this Journal. In my honest opinion, the topic is interesting enough to attract the readers' attention. Authors should only consider to add further elements to discuss, at least briefly, current challenges for ovarian stimulation in patients affected by (authors may refer to: PMID: 29274003; PMID: 31755673)

REVIEWER	Ferrero, Simone Universita degli Studi di Genova
REVIEW RETURNED	09-Jul-2021

GENERAL COMMENTS	This is an interesting and appropriately written trial that will be of great interest to physicians treating endometriosis-related infertility.

REVIEWER	Martins, Wellington Universidade de Sao Paulo Faculdade de Medicina, Department of Obstetrics and Gynecology
REVIEW RETURNED	20-Oct-2021

GENERAL COMMENTS	Introduction section Authors are completely biased in their introduction, as they want to build a rationale for performing the study. Authors state that "endometriosis is also associated with poorer IVF outcomes", but this is absolutely not true. A systematic review including more than 90 studies and more than 120,000 women observed no significant difference in live birth and clinical pregnancy rate when comparting women with and without endometriosis (1). Additionally, another review comparting evaluating the impact of endometrioma, also didn't find worse reproductive outcomes (2). Actually, data from SART shows that "women who have endometriosis in isolation, the live birth rate is

	similar or slightly higher compared with other infertility diagnoses" (3). One should also be aware that the prevalence of endometriosis increases with age, going from 3% at the age of 20-24 years until 19% at the age of 40-44 years (4). Therefore, when comparting women with vs. without endometriosis, we are actually at a very high risk of comparting older women with younger women, and any observed worse reproductive outcome in women with endometriosis should be considered with caution, since it might be caused because of the impact of age. Therefore, authors must be much more conservative in their introduction section, even though this undermines their rationale for performing the study (if women with endometriosis have similar outcomes, why using a particular intervention for this group). Randomization process is not completely clear, particularly the allocation concealment and implementation: From CONSORT: "Allocation concealment: Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned" "Implementation: Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions" Authors should also consider in their informed consent that they a negative effect of the intervention is wasting time (at least two months), which might have an impact on reproductive outcomes, particularly if considering the possibility of performing multiple IVF cycles (if each cycle takes one month, it is possible to perform 12 cycles in one year, against one 4 cycles, if waiting two months before starting ovarian stimulation). References 1. Barbosa MA, Teixeira DM, Navarro PA, Ferriani RA, Nastri CO, Martins WP. Impact of endometriosis and its staging on assisted reproduction outcome: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2014 Sep;44(3):261-78. doi: 10.1002/uog.13366. Epub 2014 Aug 13. PMID: 24639087. 2. Hamdan M, Dunselman G, Li TC, Cheong Y. The impact of endometrioma on IVF/ICSI outcomes: a systematic review and meta-analysis. Hum Reprod Update. 2015 Nov-Dec;21(6):809-25. doi: 10.1093/humupd/dmv035. Epub 2015 Jul 12. PMID: 26168799. 3. Senapati S, Sammel MD, Morse C, Barnhart KT. Impact of endometriosis on in vitro fertilization outcomes: an evaluation of the Society for Assisted Reproductive Technologies Database. Fertil Steril. 2016 Jul;106(1):164-171.e1. doi: 10.1016/j.fertnstert.2016.03.037. Epub 2016 Apr 7. PMID: 27060727; PMCID: PMC5173290. 4. Eisenberg VH, Weil C, Chodick G, Shalev V. Epidemiology of endometriosis: a large population-based database study from a healthcare provider with 2 million members. BJOG. 2018 Jan;125(1):55-62. doi: 10.1111/1471-0528.14711. Epub 2017 Jun 14. PMID: 28444957.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Antonio Simone Laganà, University of Insubria
Comments to the Author:

I read with great interest the manuscript, which falls within the aim of this Journal. In my honest opinion, the topic is interesting enough to attract the readers' attention.

Authors should only consider to add further elements to discuss, at least briefly, current challenges for ovarian stimulation in patients affected by (authors may refer to: PMID: 29274003; PMID: 31755673)

- We thank the reviewer for his interest in our study, and for referring us to these interesting references. We have incorporated both references into a more thorough discussion about current treatment and challenges in endometriosis-associated infertility.

Lines 82-88
Multiple effective treatments exist for the management of endometriosis-associated infertility, including laparoscopic destruction of endometriotic disease, ovulation induction, and intrauterine insemination [1,16,17]. While some studies show that conservative treatment alone may be effective in improving fertility for women with endometriosis and potentially helps avoid obstetrical complications associated with IVF [17], IVF remains the most direct and effective treatment for endometriosis-associated infertility, especially in patients who have failed conservative interventions.

Reviewer: 2

Dr. Simone Ferrero, Universita degli Studi di Genova
Comments to the Author:

This is an interesting and appropriately written trial that will be of great interest to physicians treating endometriosis-related infertility.

- We thank the reviewer for his supportive comments.

Reviewer: 3

Dr. Wellington Martins, Universidade de Sao Paulo Faculdade de Medicina
Comments to the Author:

Introduction section

Authors are completely biased in their introduction, as they want to build a rationale for performing the study. Authors state that "endometriosis is also associated with poorer IVF outcomes", but this is absolutely not true. A systematic review including more than 90 studies and more than 120,000 women observed no significant difference in live birth and clinical pregnancy rate when comparting women with and without endometriosis (1). Additionally, another review comparting evaluating the impact of endometrioma, also didn't find worse reproductive outcomes (2). Actually, data from SART shows that "women who have endometriosis in isolation, the live birth rate is similar or slightly higher compared with other infertility diagnoses" (3).

- We thank the reviewer for this nuanced discussion of the potential impact of endometriosis on IVF outcomes. We agree that this is an area of some controversy, with mixed results reported in the literature. In response to this reviewer's comments and concerns, we have incorporated the references that are cited in this comment, as well as additional references identified following further literature review. We have edited our introduction extensively to acknowledge the controversy in the impact of endometriosis on IVF outcomes:

Lines 89-90:
There is some evidence that endometriosis is also associated with poorer IVF outcomes, though this is controversial.

Lines 94-113:
A more recent study published in 2018 showed via retrospective comparison of 531 women with endometriosis and 737 women with unexplained subfertility found that women with endometriosis still have a 24% lower likelihood of live birth after IVF than women with unexplained infertility [19]. However, a larger retrospective study in 2017 comparing 3583 women with endometriosis and 18,833 women without endometriosis found that endometriosis was associated with fewer oocytes retrieved, but no significant difference in live birth rates [20]. Similarly, a metanalysis of 33 studies published in 2015 examined the effect of endometrioma on IVF/ICSI outcomes and also found that women with endometrioma had fewer oocytes retrieved, but similar live birth rates [21]. A large metanalysis of 78 studies published in 2014 also found a difference in number of oocytes retrieved but no significant difference in live birth rates between women with and without endometriosis [22]. It is important to note, however, that the effect of endometriosis on IVF outcomes may be underestimated, especially in more recent studies, due to a likely underdiagnosis of endometriosis in the IVF population as laparoscopy is no longer a routine practice in infertility evaluations [4]. As endometriosis commonly presents in conjunction with other infertility diagnoses (e.g. male factor, tubal factor, diminished ovarian reserve), it has been suggested that endometriosis, when associated with other barriers to fertility, contributes to poorer IVF outcomes in the general IVF population, even if this effect is not seen with endometriosis in isolation [23].

Lines 114-117:
As the association between endometriosis and poorer IVF outcomes remains biologically plausible despite mixed clinical evidence [1,13–15,23], pre-treatment with GnRH agonist therapy has been investigated as a method to improve IVF outcomes, though with mixed evidence.

Lines 122-126:
In the IVF setting, prolonged GnRH agonist treatment prior to IVF has been shown by several studies to improve fertility rates in women with advanced endometriosis [25–27], though a recent Cochrane review of 8 RCTs was unable to determine whether prolonged GnRH agonist treatment improved subsequent IVF outcomes, partly due to low quality of available evidence [28].

One should also be aware that the prevalence of endometriosis increases with age, going from 3% at the age of 20-24 years until 19% at the age of 40-44 years (4). Therefore, when comparting women with vs. without endometriosis, we are actually at a very high risk of comparting older women with younger women, and any observed worse reproductive outcome in women with endometriosis should be considered with caution, since it might be caused because of the impact of age.

- We thank the Reviewer for this important insight into one potential source of confounding when considering the effect of endometriosis on IVF outcomes. On review of existing studies on the association between endometriosis and IVF outcomes, age is a potential confounder that was accounted for in statistical analyses, though many studies also found no significant difference in age between endometriosis and non-endometriosis groups.

- While an interesting consideration, the association between age and endometriosis diagnosis is not a primary concern for our study design, as all patients recruited in our study will have a diagnosis of endometriosis, based on our inclusion criteria. We will also incorporate an age-stratified analysis in our own study.

Therefore, authors must be much more conservative in their introduction section, even though this undermines their rationale for performing the study (if women with endometriosis have similar outcomes, why using a particular intervention for this group).

- Our introduction has now been extensively edited to convey a more conservative and balanced discussion regarding the potential effect of endometriosis on IVF outcomes, as above. We thank the Reviewer for taking the time to discuss these controversies with us, as we feel our Introduction is now much improved as a result.

Randomization process is not completely clear, particularly the allocation concealment and implementation. From CONSORT: "Allocation concealment: Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned"
"Implementation: Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions"

- Randomization is described in lines 260 - 273. We have edited this section to provide more details on randomization, allocation concealment, and implementation.

Lines 266-272:
A computer-generated randomization list will be created by staff at the PREGnant Data Coordinating Center (DCC) and randomization will be performed prior to the first dose of elagolix or placebo. Randomization will have random sizes (2,4, or 6) of blocks, stratified by site and age group (<35 versus ≥35 years). Both participants and investigators will be blinded to the treatment assignment during the trial duration (except for serious safety concerns). Treatment allocation information will not be accessible to investigators (except for serious safety concerns), trial staff at the site or central laboratory personnel during the trial.

Authors should also consider in their informed consent that they a negative effect of the intervention is wasting time (at least two months), which might have an impact on reproductive outcomes, particularly if considering the possibility of performing multiple IVF cycles (if each cycle takes one month, it is possible to perform 12 cycles in one year, against one 4 cycles, if waiting two months before starting ovarian stimulation).

- While seeking ethical approval for our protocol, it was determined that because the GnRH antagonist will be administered during the routine evaluation conducted prior to the IVF cycle, and patients receiving standard IVF care normally undergo extensive laboratory testing as well as sonohysterogram and semen analysis, a two-month course of antagonist is unlikely to delay onset of IVF stimulation. Our manuscript has been edited to make this more clear.

Lines 222-223:
The GnRH antagonist will be administered during the routine evaluation conducted prior to the IVF cycle.

- A subset of patients may undergo multiple courses of antagonist vs. placebo (patients undergoing frozen embryo transfer). No more than two embryo transfers will be allowed under our study protocol, limiting administration of study drug to a maximum of three 8-week courses (one prior to IVF, and one prior to each embryo transfer). This limits the amount of potential delay experienced by patients due to their participation in this protocol. Our manuscript has been edited to make this more clear.

Lines 251-253
No more than two embryo transfers will be performed under this protocol, limiting administration of study drug to a maximum of three 8-week courses (one prior to IVF, and one prior to each embryo transfer).

- The timing of these potential delays is described in the consent form (Page 2), which is now attached.

VERSION 2 – REVIEW

REVIEWER	Martins, Wellington Universidade de Sao Paulo Faculdade de Medicina, Department of Obstetrics and Gynecology
REVIEW RETURNED	25-Dec-2021

GENERAL COMMENTS	Abstract This sentence needs to be changed in order to disclose our uncertainty (actually, I am pretty sure that endometriosis has no negative impact on IVF outcomes – all the observed difference so far are the result of important source of bias as age and surgery related decline in ovarian reserve) Lines 26-28 “Pregnancy rates are diminished in women seeing fertility treatment for endometriosis-associated infertility compared to other etiologies of infertility.” I would also ask to change the discussion Lines 43-44: “We hypothesize that GnRH antagonist pre-treatment (might) increase the live birth rate among women with endometriosis undergoing IVF-ET.” Introduction I would suggest removing lines 104-111 as they are completely speculative. If authors prefer to keep it, they must also include some important source of bias that might overestimate the effect of endometriosis on live birth. 1. Women with endometriosis are likely to be older, and there is no statistical trick that can robustly compensate this important source of bias. 2. Women with endometriosis are likely to have poor ovarian reserve due to previous surgeries that are performed to increase their fertility (but that actually only damage their ovaries). 3. Women with endometriosis are likely to have more difficult oocyte retrievals due to adhesions, resulting in fewer oocytes retrieved. Methods Randomization (lines 241-255). The allocation concealment mechanism and the implementation are still not clear. Allocation concealment mechanism: Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned.

	Implementation: Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions Sample size and power calculation “The average live birth rate for women with endometriosis undergoing IVF is estimated to be 39%…. We conservatively estimate an effect of 10% absolute improvement in live birth rate to 49% in the GnRH antagonist arm.” An absolute 10% increase (meaning a relative increase of 25%) is not conservative at all. Actually, this would be a large beneficial effect. Actually, a 5% increase would already be clinically relevant. Authors should rewrite this section in order to better explain that their study will be only sufficiently powered to detect a large effect (> 10% change LBR), not allowing to detect smaller, but still relevant changes (e.g. between 5-10%).

VERSION 2 – AUTHOR RESPONSE

Reviewer: 3
Dr. Wellington Martins, Universidade de Sao Paulo Faculdade de Medicina
Comments to the Author:

Abstract
This sentence needs to be changed in order to disclose our uncertainty (actually, I am pretty sure that endometriosis has no negative impact on IVF outcomes – all the observed difference so far are the result of important source of bias as age and surgery related decline in ovarian reserve)
Lines 26-28 “Pregnancy rates are diminished in women seeing fertility treatment for endometriosis-associated infertility compared to other etiologies of infertility.”

- We have edited this sentence to more accurately convey the uncertainty of whether endometriosis negatively impacts IVF outcomes:
- Lines 25 - 28:

While in vitro fertilization-embryo transfer (IVF-ET) successfully treats endometriosis-associated infertility, there is some evidence that pregnancy rates may be diminished in women seeing fertility treatment for endometriosis-associated infertility compared to other etiologies of infertility.

I would also ask to change the discussion
Lines 43-44: “We hypothesize that GnRH antagonist pre-treatment (might) increase the live birth rate among women with endometriosis undergoing IVF-ET.”

- This sentence has been deleted to comply with the Journal’s new formatting guidelines.

Introduction
I would suggest removing lines 104-111 as they are completely speculative. If authors prefer to keep

it, they must also include some important source of bias that might overestimate the effect of endometriosis on live birth.
1. Women with endometriosis are likely to be older, and there is no statistical trick that can robustly compensate this important source of bias.
2. Women with endometriosis are likely to have poor ovarian reserve due to previous surgeries that are performed to increase their fertility (but that actually only damage their ovaries).
3. Women with endometriosis are likely to have more difficult oocyte retrievals due to adhesions, resulting in fewer oocytes retrieved.

- We appreciate these helpful insights. At this reviewer's suggestion, we have included a discussion of the effect of adhesive disease and prior surgery on IVF outcomes, and included citations to reference the effect of surgery on ovarian reserve and folliculogenesis. We also agree that the technical effect of adhesive disease on oocyte retrievals is an important consideration and have chosen to incorporate this into our discussion, but we were unable to find citations on the effect of adhesive disease on oocyte retrievals.

- After careful consideration, we did not include a discussion of the potential uncorrected effect of age. In the study by Senapati et al that is referenced in this paragraph, the mean age of patients with isolated endometriosis (34.6 ± 4.1 years) and patients with endometriosis and an additional infertility diagnosis (35.3 ± 4.4) was younger (but not significantly so) than that of patients with unexplained infertility (35.7 ± 4.1), and significantly younger than that of patients with all other causes of infertility (36.0 ± 4.9). Thus, in this sample, the effects of endometriosis on IVF outcomes are unlikely to be confounded by the effects of advanced age in the referenced study, though this may be a valid concern in other studies published on this matter. We agree that age should always be considered an important confounder when performing studies on endometriosis and fertility, and a careful age-adjusted analysis remains critical.

- Lines 117-123:

This finding may be due to a primary effect of endometriosis on reproductive biology, but may also be secondary to epidemiologic or iatrogenic factors associated with an endometriosis diagnosis: greater exposure to prior gynecologic surgery resulting in ovarian injury, diminished reserve, or impaired folliculogenesis) [24–26], or an effect of subsequent adhesive disease on the technical difficulty of oocyte retrievals. These factors, however, have not been well studied as potential mechanisms by which endometriosis may compromise IVF outcomes.

Methods
Randomization (lines 241-255).
The allocation concealment mechanism and the implementation are still not clear.
Allocation concealment mechanism: Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned.
Implementation: Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions

- We have added clarifying details to this section:

- Lines 258 – 269:

A computer-generated randomization list will be created by staff at the PREGnant Data Coordinating Center (DCC) and randomization will be performed prior to the first dose of elagolix or placebo. Randomization will have random sizes (2,4, or 6) of blocks, stratified by site and age group (<35 versus ≥35 years). The randomization list will not be available to any person involved in the conduct and evaluation of the trial until the trial is complete and database is declared clean and is released by the DCC. Randomization and treatment allocation will be initiated by study staff accoding to the randomization list following enrollment and prior to the first dose of elagolix or placebo, but participants, investigators, trial staff, and central laboratory personnel will be blinded to the treatment assignment during the trial duration (except for serious safety concerns). The assigned treatment (GnRH antagonist vs. placebo) applied during the fresh cycle will also be used for subsequent frozen embryo transfers resulting from the initial 'fresh' egg retrieval cycle.

Sample size and power calculation
"The average live birth rate for women with endometriosis undergoing IVF is estimated to be 39%…. We conservatively estimate an effect of 10% absolute improvement in live birth rate to 49% in the GnRH antagonist arm."
An absolute 10% increase (meaning a relative increase of 25%) is not conservative at all. Actually, this would be a large beneficial effect. Actually, a 5% increase would already be clinically relevant. Authors should rewrite this section in order to better explain that their study will be only sufficiently powered to detect a large effect (> 10% change LBR), not allowing to detect smaller, but still relevant changes (e.g. between 5-10%).

- We thank the Reviewer for this astute observation, and acknowledge that our study will be underpowered to detect these smaller effects. Our discussion has been edited to acknowledge this important point:

- Lines 303-309

Using 386 participants per arm (N=772) would provide an alpha of 0.05 and power of 80% to detect an effect of 10% absolute improvement in live birth rate to 49% in the GnRH antagonist arm. This effect is conservatively estimated from prior randomized trials using GnRH agonists [28–30], and what investigators deemed to be sufficient to recommend the routine use of GnRH antagonists in IVF protocols for this population. However, we acknowledge that the study may be underpowered to detect smaller but still relevant effects (5-10% improvement).

VERSION 3 – REVIEW

REVIEWER	Martins, Wellington Universidade de Sao Paulo Faculdade de Medicina, Department of Obstetrics and Gynecology
REVIEW RETURNED	05-Mar-2022

GENERAL COMMENTS	I am still concerned about the allocation concealment mechanism. "A computer-generated randomization list will be created by staff at the PREGnant Data Coordinating Center (DCC) and randomization will be performed prior to the first dose of elagolix or placebo. Randomization will have random sizes (2,4, or 6) of blocks, stratified by site and age group (<35 versus ≥35 years). The randomization list will not be available to any person involved in the conduct and evaluation of the trial until the trial is complete and database is declared clean and is released by the DCC.

	Randomization and treatment allocation will be initiated by study staff accoding to the randomization list following enrollment and prior to the first dose of elagolix or placebo, but participants, investigators, trial staff, and central laboratory personnel will be blinded to the treatment assignment during the trial duration (except for serious safety concerns). The assigned treatment (GnRH antagonist vs. placebo) applied during the fresh cycle will also be used for subsequent frozen embryo transfers resulting from the initial 'fresh' egg retrieval cycle." In my opinion, it is still not completely clear how the participants will be assigned. For example? are the placebo and elagolix completely similar and consecutively numbered and only with the list would be possible to know who was using placebo and who was using elagolix? In this case, would women below 35 years and those after 35 years receive the intervention by the order of receiving the first dose of the intervention?

VERSION 3 – AUTHOR RESPONSE

Reviewer: 3
Dr. Wellington Martins, Universidade de Sao Paulo Faculdade de Medicina
Comments to the Author:

I am still concerned about the allocation concealment mechanism.
In my opinion, it is still not completely clear how the participants will be assigned.
For example? are the placebo and elagolix completely similar and consecutively numbered and only with the list would be possible to know who was using placebo and who was using elagolix? In this case, would women below 35 years and those after 35 years receive the intervention by the order of receiving the first dose of the intervention?

- Thank you for this comment. We have clarified that randomization sequences will be independently and randomly generated within each site and age strata, using random block sizes. That is, separate, independent randomization sequences will be generated for women < 35 years and ≥ 35 years at each study site. Thus, treatment allocation would not be able to be deduced by order of enrollment or allocation without access to the randomization list. The manuscript text has been updated to clarify this point.

Lines 251-257:
A computer-generated randomization list will be created by staff at the PREGnant Data Coordinating Center (DCC) and randomization will be performed prior to the first dose of elagolix or placebo. Randomization will have random sizes (2,4, or 6) of blocks, stratified by site and age group (<35 versus ≥35 years). Randomization sequences within each study site and age stratum will be generated randomly and independently. The randomization list will not be available to any person involved in the conduct and evaluation of the trial until the trial is complete and database is declared clean and is released by the DCC.