PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The effectiveness of modified downregulation for women with moderate and severe adenomyosis of the uterus prior to frozen thawed embryo transfer (MODA) study protocol: a pragmatic randomised-controlled trial.
AUTHORS	Latif, Sania; Wattar, Bassel H.Al; Balachandren, Neerujah; Lukaszewski, Tomasz; Saridogan, Ertan; Yasmin, Ephia; Serhal, Paul; Mavrelos, Dimitrios

VERSION 1 – REVIEW

REVIEWER	Tremellen, Kelton Flinders University
REVIEW RETURNED	25-Mar-2021

GENERAL COMMENTS	This paper describes the trial protocol for a non-blinded RCT allocating women with moderate to severe adenomyosis (diagnosed on a 7 point scale) to either a short-down regulation (1 week) HRT frozen embryo cycle or a long down regulation (6 week) HRT cycle with subsequent transfer of a frozen embryo. The theory behind this is based on the fact that GnRH agonist long down regulation produces a reduction in ovarian estrogen production that inactivates adenomyosis. The benefit of this approach in an IVF context was first reported by Tremellen and Russell (2011) a decade ago. Interestingly, this seminal paper is not referenced- an obvious omission that should be corrected. Overall, the paper is well written and most aspects of a RCT design are adequate. I would suggest the following aspects could be improved: 1. As it is neither necessary, nor standard practice in most IVF centres to use down-regulation HRT FET cycles in ovular women, why did the authors make this as their control comparator. It is a lot more patient friendly for these women to undergo a natural ovular cycle FET without any exposure to GnRH agonist. There is data suggesting GnRH receptors in adenomyosis, so it seems illogical to expose a control to GnRH agonist at all. This is a major weakness for me as it means the MODA study will only be able to anser what duration of GnRH agonist works better than the other, not no agonist natural cycle (standard default treatment in FET). 2. I am surprised that the authors are enrolling women up to 42 years of age. Embryo aneuploidy rates are so high post 40 that without PGT-A testing, the implantation potential in any FET will be low and may make this study underpowered if a large number

	of older women are enrolled. I would have thought a PGT-A confirmed euploid embryo in storage would be a requirement for entry of older patients in this trial. Its certainly my clinical practice before doing down-regulation therapy in older adenomyosis patients. 3. I am puzzled why sperm quality and ovarian reserve markers (AMH, FSH) are inclusion/exclusion criteria. Surely the production of a high-quality embryo for cryo-storage, preferably a blastocyst, is the only relevant criteria for enrolment- irrespective of sperm quality or ovarian reserve status. 4. I could not find any reference to the type of embryo transfer being conducted. Is it cleavage stage or blastocyst. Can embryos undergo genetic testing? If so, how is this all accounted for in the randomisation process? 5. The primary outcome here is clinical pregnancy, not live birth. As adenomyosis is associated with a higher risk of euploid miscarriage, surely a live birth is the ideal primary end point? 6. Long periods of down regulation produce significant side effects on the women (headaches, hot flushes, irritability, poor cognition), yet now of these are considered as secondary study end points. Why not? 7. Adjuvants such as prednisolone are commonly used in adenomyosis treatment given that studies have linked adenomyosis with endometrial inflammation. Does this study allow the use of such adjuvants? 8. On page 18, lines 21-32 outlining the sample size, I feel the authors have made an error. Here they state that the clinical pregnancy rate in mild adenomyosis is 22.9% v 42.7% in moderate/severe adenomyosis. Are they really saying that pregnancy rates get better with an increase in severity of adenomyosis disease? I am sure they means the reverse.

REVIEWER	Pirtea, Paul Hopital Foch, IVF
REVIEW RETURNED	13-May-2021

GENERAL COMMENTS	The authors did a great job but I have some concerns: Actually the actual real down regulation and estrogen depravation is in the modified group of 14 days. From the data that is available we know now that a 14 days. Also the real limitations of the study are not at all presented.

REVIEWER	Wang, Junxia Nanjing University
REVIEW RETURNED	06-Jun-2021

GENERAL COMMENTS	1.The description of BMI, AMH and FSH in the inclusion criteria does not provide a unit. 2.The authors need to provide references for the inclusion criteria of AMH>5.4 and AFC>4. 3.The exclusion criteria are not adequate enough. It seems necessary to add the exclusion of hydrosalpinx, severe endometriosis and endometrial polyps or other lesions.

	4.The number of frozen embryos available for transfer might be independent of GnRHa pretreatment and should not be considered as a key secondary outcome. 5.In the introduction, the authors mentioned that whether GnRHa pretreatment could improve IVF/ICSI outcome was still controversial (references: 9, 10 and 11). Among these three references, only the first one is about frozen embryo transfer cycles, and the other two articles are mainly about fresh embryo transfer cycles. It would be better to provide more references for the frozen embryo transfer cycle of adenomyosis patients. 6.In addition, the duration of GnRHa pretreatment mentioned in the above references was different, which is mostly longer than 6 weeks. Previous studies have reported that GnRHa pretreatment for 3-6 months can significantly improve the clinical pregnancy rate of patients with endometriosis in IVF/ICSI cycles. At the same time, our previous study also found that GnRHa pretreatment for at least 3 months could improve the clinical outcomes of patients with adenomyosis in IVF/ICSI cycles. Therefore, whether the 6-week pretreatment is too short to show the real effect of GnRHa, or more theoretical or literature support is needed for the duration of 6 weeks. 7.The authors set up a short-term pretreatment project with GnRHa as the control group. This project is rarely reported for HRT-FET cycles. The authors should provide more evidence for chosing this project as the control group. 8.There seems to be a typographical error at the end of page 13. 9.In the aspect of sample size, the authors seem to have a wrong description: Our previous observational studies suggest a clinical pregnancy rate of 22.9% in women with mild adenomyosis compared to 42.7% with moderate/severe adenomyosis.

VERSION 1 – AUTHOR RESPONSE

Reviewer Reports:

Reviewer: 1

Dr. Kelton Tremellen, Flinders University

Author's response:

- We agree with the reviewer and have updated our manuscript to include reference to this paper on page 8 and in the references item 9.

Overall, the paper is well written and most aspects of a RCT design are adequate. I would suggest the following aspects could be improved:

1. As it is neither necessary, nor standard practice in most IVF centres to use down-regulation HRT FET cycles in ovular women, why did the authors make this as their control comparator. It is a lot more patient friendly for these women to undergo a natural ovular cycle FET without any exposure to GnRH agonist. There is data suggesting GnRH receptors in adenomyosis, so it seems illogical to expose a control to GnRH agonist at all. This is a major weakness for me as it means the MODA study will only be able to answer what duration of GnRH agonist works better than the other, not no agonist natural cycle (standard default treatment in FET).

Author's response:

- We agree with the reviewer that this trial evaluates two different durations of downregulation. In our setting we use a mid-luteal phase downregulation protocol as standard practice for frozen thawed embryo transfer to aid scheduling of treatment and reduce cancellation rates. We would welcome an additional trial comparing natural cycle frozen thawed embryo transfer and mid-luteal phase downregulation protocol for frozen thawed embryo transfer cycles. We have not made any changes to the manuscript in relation to this comment.

2. I am surprised that the authors are enrolling women up to 42 years of age. Embryo aneuploidy rates are so high post 40 that without PGT-A testing, the implantation potential in any FET will be low and may make this study underpowered if a large number of older women are enrolled. I would have thought a PGT-A confirmed euploid embryo in storage would be a requirement for entry of older patients in this trial. Its certainly my clinical practice before doing down-regulation therapy in older adenomyosis patients.

Author's response:

- We agree with the reviewer in older women PGT-A is a helpful adjunct. However, PGT-A is not without controversy and it is not offered on the NHS in the UK, which is beyond our control. It would be difficult to offer PGT-A in a subset of women and would affect the feasibility of the trial as some women would have no embryo to transfer. Offering PGT-A only to a subset would create a confounding variable. In our practice the proportion of women above the age of 40 is small and through randomisation should be balanced between the two groups. We have not made any change to our manuscript in relation to this comment.

3. I am puzzled why sperm quality and ovarian reserve markers (AMH, FSH) are inclusion/exclusion criteria. Surely the production of a high-quality embryo for cryo-storage, preferably a blastocyst, is the only relevant criteria for enrolment- irrespective of sperm quality or ovarian reserve status.

Author's response:

- IVF funding in the NHS involves ovarian reserve criteria and is beyond our control. We agree with the reviewer that embryo quality is of importance in this trial. Our practice is to perform blastocyst transfer for all our patients with a minimum morphological quality of B-C. This should ensure that variability from embryo quality variation is controlled. We have updated our manuscript to clarify the type of embryo transfer being performed on page 13.

4. I could not find any reference to the type of embryo transfer being conducted. Is it cleavage stage or blastocyst. Can embryos undergo genetic testing? If so, how is this all accounted for in the randomisation process?

Author's response:

- As above, we are only going to transfer B-C or above blastocyst embryos. We have updated our manuscript to clarify the type of embryo transfer being performed on page 13. Genetic testing with PGT-A is not offered on the NHS in the UK.

5. The primary outcome here is clinical pregnancy, not live birth. As adenomyosis is associated with a higher risk of euploid miscarriage, surely a live birth is the ideal primary end point?

Author's response:

- We agree with the reviewer that live birth is an important outcome here and we will report this secondarily. Our sample size calculation was based on clinical pregnancy and in the interest of publicising our results in the shortest timeframe possible we have chosen clinical pregnancy as our primary outcome. We have not made anchange to our manuscript in relation to this comment.

6. Long periods of down regulation produce significant side effects on the women (headaches, hot flushes, irritability, poor cognition), yet now of these are considered as secondary study end points. Why not?

Author's response:

- Our secondary outcomes will include the frequency and severity of side effects as suggested. We have updated the manuscript to clarify this on page 2 and 10.

7. Adjuvants such as prednisolone are commonly used in adenomyosis treatment given that studies have linked adenomyosis with endometrial inflammation. Does this study allow the use of such adjuvants?

Author's response:

- We do not use prednisolone for the treatment of adenomyosis related inflammation. We have not made any change to our manuscript in relation to this comment.

8. On page 18, lines 21-32 outlining the sample size, I feel the authors have made an error. Here they state that the clinical pregnancy rate in mild adenomyosis is 22.9% v 42.7% in moderate/severe adenomyosis. Are they really saying that pregnancy rates get better with an increase in severity of adenomyosis disease? I am sure they means the reverse

Author's response:

- Thank you for pointing this out, we have corrected this error in the manuscript on page 16.

Reviewer: 2

Dr. Paul Pirtea, Hopital Foch

Author's response:

- We have expanded on the limitations of the trial as suggested by this reviewer in the manuscript on page 17. In particular we now discuss the potential for confounding from undiagnosed endometriosis as not all patients with adenomyosis will undergo surgical diagnostic procedures. We have also expanded on the potential for variability between centres on the diagnosis of adenomyosis by ultrasound and the mitigating processes; we have only included centres with an expert ultrasound operator and will review ultrasound images to ensure quality of diagnosis.

Reviewer: 3

Dr. Junxia Wang, Nanjing University

1.The description of BMI, AMH and FSH in the inclusion criteria does not provide a unit.

Author's response:

- Thank you, we have updated the manuscript to include units on page 2 and 9.

2.The authors need to provide references for the inclusion criteria of AMH>5.4 and AFC>4.

Author's response:

- Thank you, we have updated the manuscript on page 9, to include a reference to National Institute for Health and Care Excellence guidelines for this and updated the manuscript in the references, item 15.

3.The exclusion criteria are not adequate enough. It seems necessary to add the exclusion of hydrosalpinx, severe endometriosis and endometrial polyps or other lesions.

Author's response:

- We agree with the reviewer that untreated hydrosalpinges are a criteria for exclusion and have updated the manuscript on page 2 and 9 to reflect this.
- We will record the presence of coexisting endometriosis diagnosed by surgery or ultrasound and will perform a sub-analysis to assess the impact of this variable. This is now further highlighted in our discussion page 17.
- We agree that untreated endometrial polyps are a criteria for exclusion and have updated our manuscript to reflect this on page 2 and 9.

4.The number of frozen embryos available for transfer might be independent of GnRHa pretreatment and should not be considered as a key secondary outcome.

Author's response:

- We agree with the reviewer and have updated our manuscript on page 2 and 9 to reflect this.

5. In the introduction, the authors mentioned that whether GnRHa pretreatment could improve IVF/ICSI outcome was still controversial (references: 9, 10 and 11). Among these three references, only the first one is about frozen embryo transfer cycles, and the other two articles are mainly about fresh embryo transfer cycles. It would be better to provide more references for the frozen embryo transfer cycle of adenomyosis patients.

Author's response:

- We agree with the reviewer and have updated our manuscript on page 8 to include further references for frozen embryo transfer cycles (10-14).

6.In addition, the duration of GnRHa pretreatment mentioned in the above references was different, which is mostly longer than 6 weeks. Previous studies have reported that GnRHa pretreatment for 3-6 months can significantly improve the clinical pregnancy rate of patients with endometriosis in IVF/ICSI cycles. At the same time, our previous study also found that GnRHa pretreatment for at least 3 months could improve the clinical outcomes of patients with adenomyosis in IVF/ICSI cycles. Therefore, whether the 6-week pretreatment is too short to show the real effect of GnRHa, or more theoretical or literature support is needed for the duration of 6 weeks.

Author's response:

- We agree that previous studies have shown improved reproductive outcomes using downregulation for time periods between 6 weeks and 6 months, and it would be relevant to perform a prospective randomised trial to evaluate downregulation for a period of 3 months prior to frozen embryo transfer. We are evaluating downregulation for a period of 6 weeks prior to frozen embryo transfer based on evidence from the retrospective study by Niu et al. which showed increased clinical pregnancy rates using a similar protocol in frozen thawed embryo transfer cycles. Another factor in our setting is ensuring the acceptability of the protocol to our patient population, due to the 5 weeks increased treatment time in the modified downregulation arm; many women are under time constraints as a result of age limits to IVF funding in the public care system in the UK. A further consideration is the need to balance the potential negative impact of using prolonged downregulation with GnRH analogue for extended periods of time on endometrial preparation as a result of endometrial suppression. We have not updated the manuscript in relation to this comment.

7.The authors set up a short-term pretreatment project with GnRHa as the control group. This project is rarely reported for HRT-FET cycles. The authors should provide more evidence for chosing this project as the control group.

Author's response:

- In our setting a mid-luteal phase downregulation protocol is used as standard practice for frozen thawed embryo transfer to aid scheduling of treatment and reduce cancellation rates. We would welcome an additional trial comparing natural cycle frozen thawed embryo transfer and mid-luteal phase downregulation protocol for frozen thawed embryo transfer cycles. We have not updated the manuscript in relation to this comment.

8.There seems to be a typographical error at the end of page 13.

Author's response:

- We have amended this.

9.In the aspect of sample size, the authors seem to have a wrong description: Our previous observational studies suggest a clinical pregnancy rate of 22.9% in women with mild adenomyosis compared to 42.7% with moderate/severe adenomyosis.

Author's response:

- Thank you for pointing this out, we have corrected this in the manuscript on page 16.

VERSION 2 – REVIEW

REVIEWER	Wang, Junxia Nanjing University
REVIEW RETURNED	31-Jul-2021

GENERAL COMMENTS	1.The unit of FSH on pages 12 and 45 is still incorrect (<8.9 iU), please modify it. 2.Regarding the sixth question, I think this is the limitation of your research, and I suggest that it be fully discussed in the discussion.In addition, I cannot fully agree with the author's explanation. For "A further consideration is the need to balance the potential negative impact of using prolonged downregulation with GnRH analogue for extended periods of time on endometrial preparation as a result of endometrial suppression.", does this explanation have a theoretical basis or reported in the literature?

VERSION 2 – AUTHOR RESPONSE

Reviewer: 3
Dr. Junxia Wang, Nanjing University

1.The unit of FSH on pages 12 and 45 is still incorrect (<8.9 iU), please modify it.

Author's response:

- Thank you for pointing this out, we have corrected the unit of FSH to < 8.9 IU/L on page 9 of the manuscript.

2. Regarding the sixth question, I think this is the limitation of your research, and I suggest that it be fully discussed in the discussion. In addition, I cannot fully agree with the author's explanation. For "A further consideration is the need to balance the potential negative impact of using prolonged downregulation with GnRH analogue for extended periods of time on endometrial preparation as a result of endometrial suppression.", does this explanation have a theoretical basis or reported in the literature?

Author's response:

- Thank you for your comment, we have expanded our discussion on page 17 of the manuscript to include the rationale for the duration of downregulation that we have chosen to evaluate. In particular, we clarify that the effectiveness of prolonged downregulation has not yet been established and a measured approach is required. We also include reference to the retrospective study in which 3 months downregulation with GnRH analogue prior to fresh embryo transfer found a reduction in clinical pregnancy rates, with a significantly lower endometrial thickness in the pre-treatment group that did not fall pregnant (Chen et al., 2020).

- There is a theoretical basis for why prolonged GnRH analogue could have a negative effect on endometrial preparation and some evidence to support this in the literature. Administration of a long-acting depot formulation of GnRH analogue gives rise to an initial flare effect, followed by downregulation

of the hypothalamic pituitary ovarian axis and a hypoestrogenic state 1-3 weeks later (Kumar et al., 2014). The initial downregulation effect is causd by desensitisation and the sustained response results from loss of GnRH receptors and the uncoupling of the receptor from its effector system. There is evidence that sustained exposure to high concentrations of GnRH during the initial flare can reduce the response of oestrogen receptors to subsequent stimulation (Ortmann et al., 2002). There is also evidence that GnRH analogues can cause prolonged suppression (Filicori et al., 1996). Other studies have shown that long GnRH agonist protocols can produce a delay of endometrial receptivity (Ruan et al. 2006, Horcajadas 2008, Van Vaerenbergh 2009).

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- Ruan HC, Zhu XM, Luo Q, Liu AX, Qian YL, Zhou CY, Jin F, Huang HF, Sheng JZ. Ovarian stimulation with GnRH agonist, but not GnRH antagonist, partially restores the expression of endometrial integrin beta3 and leukemia-inhibitory factor and improves uterine receptivity in mice. Hum Reprod 2006; 21:2521–2529.

- Horcajadas J, Minguez P, Dopazo J, Esteban F, Dominguez F, Giudice L, Pellicer A, Simon C. Controlled ovarian stimulation induces a functional genomic delay of the endometrium with potential clinical implications. J Clin Endocrinol Metab 2008; 93:4500–4510.

- Van Vaerenbergh I, Van Lommel L, Ghislain V, In’ t Veld P, Schuit F, Fatemi HM, Devroey P, Bourgain C. In GnRH antagonist/rec-FSH stimulated cycles, advanced endometrial maturation on the day of oocyte retrieval correlates with altered gene expression. Hum Reprod 2009; 24: 1085–1091.