PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Comparison of a “freeze all” strategy including GnRH agonist trigger versus a “fresh transfer” strategy including hCG trigger in assisted reproductive technology (ART) – A study protocol for a randomised controlled trial
AUTHORS	Stormlund, Sacha; Løssl, Kristine; Zedeler, Anne; Bogstad, Jeanette; Prætorius, Lisbeth; Nielsen, Henriette; Bungum, Mona; Skouby, Sven; Mikkelsen, Anne Lis; Andersen, Anders; Bergh, Christina; Humaidan, Peter; Pinborg, Anja

VERSION 1 - REVIEW

REVIEWER	Cornelis B Lambalk VU University Medical Center, Amsterdam, The Netherlands
REVIEW RETURNED	15-Feb-2017

GENERAL COMMENTS	Stormlund et al submitted a study protocol of an ongoing multi center double blinded international study that will be completed in May 2018 comparing ongoing pregnancy rate after the first embryo transfer in IVF/ICSI in patient randomized for either undergoing embryo transfer after when all embryos frozen and transferred in a later cycle of after immediate fresh transfer of a single blastocyst. In the “Freeze all” arm patients undergo ovulation triggering with a GnRH analogue whereas triggering in the fresh cycle will be done with hCG. In both arms prevention of premature luteinization will be done with fixed started GnRH antagonist. The manuscript is a solid description of the running study and is as such representative. It would help if the authors could end the introduction by arguing why the protocol should be published. Since the study is ongoing any of the following comments shall not lead to a change of its design: 1) A major shortcoming is that the study deals with 2 variables. In the end it will remain unclear which of the two variables have contributed to possible differences in primary outcome result. It is argued that this is a limitation of the study. See last point page 4. The investigators chose for this strategy in order to evaluate the freeze all/fresh comparison in a so called OHSS free situation. Which by the way doesn’t exist. There are several reports now on OHSS even when antagonist and agonist triggering were applied. But by doing so they will never be able to answer the principal question namely whether freeze all versus fresh ET will be of beneficial influence on OGP. This at cost of, as chosen, a secondary endpoint namely OHSS occurrence. Mistakenly not mentioned in the list of secondary objectives on page 5 but mentioned in table 3. 2) I consider the chosen primary endpoint as secondary: the ongoing pregnancy rate after the first blastocyst transfer. The bottom line is

	that the only clinically relevant final result of the treatment in both arms is of course their first mentioned secondary endpoint namely the cumulative live birth rate (or ongoing pregnancy may be acceptable as well) after one complete cycle including all frozen thawed transferred embryos. 3) Some minor point are furthermore: a. When the study is completed in May 2018 will that be when results are known for the primary endpoint? b. Why only one Bologna poor responder criterion for exclusion and not also for example one of the other criteria such as > 38 yrs or previous poor response. c. Why is the doctor with his/her selected starting dose (and what contributed to the choice?) part of the treatment variability? I assume that each clinic has its own protocol and why was that not taken as starting point? How was this dose selection put into the randomization program exactly? d. I miss MZ twinning rate as secondary endpoint. e. What instructions do patients get with regard to intercourse around time of frozen/thawed ET? f. Beta-hCG is not the type of hCG we measure in pregnant women. It is the intact heterodimer of hCG. g. Page 9 line 22: Women as well as male partners will answer a questionnaire …. Are couples included with a female partner? I suggest to address the points made extensively in the discussion.

REVIEWER	Coetzee, Kevin Antalya IVF, Turkey
REVIEW RETURNED	20-Feb-2017

GENERAL COMMENTS	A “Freeze-all” versus a “Fresh embryo transfer”-strategy in assisted reproductive technology (ART): Study protocol for a multicentre randomized controlled trial Stormlund et al BMJ open This study aims to compare ongoing pregnancy and live birth rates between a freeze-all strategy with GnRH agonist triggering versus hCG trigger and fresh embryo transfer in a multicentre randomized. A multinational multicentre randomized control trial The authors need to consistently describe the study as the comparison of two strategies, one that includes an hCG trigger and fresh ET and the other a GnRH trigger and FET. Therefore the title of the study may be misleading. Although the study as described in the title and objectives only compares two IVF strategies in terms of ongoing pregnancy, it is obvious that the study includes multiple sub-studies. The authors may need to more deliberately express this intention. Because this study in its entirety (full duration) includes the collection of live birth outcomes and beyond, it seems unusual that the primary objective has been written as follows: The primary endpoint is to compare ongoing pregnancy rates in the two treatment groups after the first single blastocyst transfer.

	The authors need to fully describe how the following 'fresh embryo transfer' subgroup will be analyzed; If > 18 follicles with a diameter >11 mm are observed, GnRH agonist triggering should be used and all blastocysts frozen (Papanikolaou et al., 2011). In multinational multicentre studies, heterogeneity is always a concern and a significant source of bias. Even though the randomization will be performed according to trial site, the study can only be strengthened if the outcomes from each centre prior to the start of the study are shown to be consistent.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1 Cornelius B Lambalk, VU University Medical Center, Amsterdam, The Netherlands

Thanks to Reviewer 1 for all the relevant comments.

Comments to authors:

Stormlund et al submitted a study protocol of an ongoing multicenter double blinded international study that will be completed in May 2018 comparing ongoing pregnancy rate after the first embryo transfer in IVF/ICSI in patient randomized for either undergoing embryo transfer after when all embryos frozen and transferred in a later cycle of after immediate fresh transfer of a single blastocyst. In the “Freeze all” arm patients undergo ovulation triggering with a GnRH analogue whereas triggering in the fresh cycle will be done with hCG. In both arms prevention of premature luteinization will be done with fixed started GnRH antagonist.
The manuscript is a solid description of the running study and is as such representative.
It would help if the authors could end the introduction by arguing why the protocol should be published.

Thanks for this comment. We have now added a section to the ending of the introduction on why this protocol should be published (Introduction: P5, L105-112, clean version):

(Introduction: P5, L105-112, clean version): OHSS is one of the most severe side effects of ART and is potentially life threatening. The present protocol describes a randomised trial assessing a new ART treatment strategy, where OHSS can be almost completely avoided. The results are very important as the majority of our patients could avoid the OHSS risk by applying the “GnRH agonist and freeze-all” strategy, maybe even with a higher chance of pregnancy. This concept has not been assessed before, and should relevantly be considered when planning studies investigating the freeze-all strategy underlining the need for large multicentre randomised controlled trials exploring the GnRH agonist and freeze-all strategy in a broad population of ART patients.

Since the study is ongoing any of the following comments shall not lead to a change of its design:

1) A major shortcoming is that the study deals with 2 variables. In the end it will remain unclear which of the two variables have contributed to possible differences in primary outcome result. It is argued that this is a limitation of the study. See last point page 4. The investigators chose for this strategy in order to evaluate the freeze all/fresh comparison in a so called OHSS free situation. Which by the way doesn’t exist. There are several reports now on OHSS even when antagonist and agonist triggering were applied. But by doing so they will never be able to answer the principal question namely whether

freeze all versus fresh ET will be of beneficial influence on OGP. This at cost of, as chosen, a secondary endpoint namely OHSS occurrence. Mistakenly not mentioned in the list of secondary objectives on page 5 but mentioned in table 3.

Thank you very much for commenting on this. We recognise the concerns raised by the reviewer. The study setup, including the use of a GnRH-agonist in the freeze-all treatment arm has been discussed extensively within the research team and was ultimately chosen to fully investigate the potential of the "complete" freeze-all strategy. We agree that it is not essentially possible to create an impervious OHSS-free strategy, as this correctly stated, does not exist. However, we find it reasonable to include a GnRH agonist trigger in the freeze-all treatment arm, as this approach represents the overall treatment strategy imposing the least possible risk of developing OHSS making the overall risk of developing OHSS extremely low in this group (Engmann et al., 2008.; Humaidan et al., 2011). In a clinical setting with an implementation of an elective freeze-all strategy where all embryos are preliminary destined to be frozen and subsequently thawed, it does not seem ideal to use an hCG-trigger in this group, knowing that the embryos are not meant for transfer until a following cycle. Furthermore, there are currently no studies implicating adverse effects on either oocyte and embryo quality after the use of a GnRH agonist (Engmann et al., 2016). For this reason, we consider it very relevant to investigate the freeze-all strategy including GnRH agonist trigger in a RCT setting. It could be argued that in a study exploring the "complete freeze-all strategy", an open randomisation setting, allowing gonadotropin dosing being decided with the beforehand knowledge of all embryos being frozen and not transferred in the stimulated cycle, but as this being the first ever RCT of a freeze-all strategy including GnRH agonist trigger, the double-blinded design was chosen to minimize differences between the two groups.

In relation to the abovementioned discussion, the following has been added to the manuscript (Discussion: P14-15, L327-346, clean version):

(Discussion: P14-15, L327-346, clean version): Evidently, we are unable to distinguish between the effect of the GnRH-agonist trigger and the effect of elective freeze-all, when both are included in the freeze-all treatment arm. The present study therefore compares an 'OHSS-free' freeze-all strategy including GnRH agonist trigger with a fresh transfer strategy with hCG trigger. In both treatment arms individualized gonadotropin dosing is used with the possibility of conversion to GnRH agonist trigger and segmentation in case of risk of OHSS development in the fresh embryo transfer group. Individualized gonadotropin dosing based on female age and weight, antral follicle count, AMH and results of previous COH cycles is applied, as this is the standard treatment approach used routinely in all of the participating clinics. The AMH cut-off value at 6.28 pmol/L (Roche Elecsys assay) corresponding to the Bologna criteria for poor ovarian response was chosen to have a reasonable chance of the patient ending up with at least one usable blastocyst after aspiration. It could be argued that an open randomisation, rather than a double-blinded study design, would allow a better exploration of the concept as higher gonadotropin doses and more oocytes could be safely aimed for in the freeze-all group. However, as this is the first RCT of a freeze-all strategy including GnRH agonist trigger, a double-blinded design was chosen to minimize differences between the two treatment arms and gonadotropin dosing is decided upon independently of allocation to treatment group, as this is done prior to randomisation. In addition, even though a strategy combining GnRH agonist trigger and freeze-all is near OHSS free, increasing gonadotropin dosing would nonetheless add a potential risk of early OHSS in the patients.

Engmann, L., DiLuigi, A., Schmidt, D., Nulsen, J., Maier, D., Benadiva, C. The use of gonadotropin-relasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. Fertil Steril 2008; 89, 84-91

Engmann, L., Benadiva, C., Humaidan, P. GnRH agonist trigger for the induction od oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis. Reproductive BioMedicine Online 2016; 32, 274-285.

Humaidan, P., Kol, S., Papanikolaou, E.G., Copenhagen Gn, R.H.A.T.W.G. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? Hum. Reprod. Update 2011; 17, 510-524.

Furthermore, as correctly noticed by the reviewer, OHSS was mistankenly missing from the list of secondary objectives and has now been added on P5, L130, clean manuscript version.

2) I consider the chosen primary endpoint as secondary: the ongoing pregnancy rate after the first blastocyst transfer. The bottom line is that the only clinically relevant final result of the treatment in both arms is of course their first mentioned secondary endpoint namely the cumulative live birth rate (or ongoing pregnancy may be acceptable as well) after one complete cycle including all frozen thawed transferred embryos.

Thank you for this comment. The primary endpoint of the study has mistakenly not been asserted unambiguously and to properly clarify this we have rephrased this as needed to ongoing pregnancy rate per randomised patient after the first potential blastocyst transfer. This has furthermore been clarified in the latest protocol (version 3, 18022017), which has also been attached for this resubmission. We sincerely apologize for this confusion.

However, we fully agree that the ideal primary endpoint is the cumulative live birth rate after one complete cycle. Unfortunately it has been necessary within the appointed timeframe of the received funding from the Reprounion program, allowing us a time limit of 3 years to complete the study, to compromise on the number of patients possible to enroll within this period. The power calculation indicating a necessary 424 patients (212 in arch treatment arm) required to have an 80 % chance of detecting an increase in the primary outcome measure from 30 % to 43 % has therefore been calculated on ongoing pregnancy rate per randomised patient after the first potential blastocyst transfer. Nevertheless, we fully agree that the cumulative live birth rate is a very important endpoint, and this is also planned to be determined as secondary endpoint.

In addition, we think that the primary endpoint of ongoing pregnancy rate per randomised patient after the first potential blastocyst transfer of this study is important, as we might find a “dilution” of the effect of the freeze-all strategy in cumulated rates due to the fact that gonadotropin dosing is decided upon equally in the two treatment groups independently of treatment allocation, and the number of aspirated oocytes is therefore expected to be the same in both groups. We have added a section to the manuscript addressing this (Discussion: P15 L347-352, clean version):

(Discussion: P15 L347-352, clean version): The primary endpoint of this study is to investigate ongoing pregnancy rates per randomised patient after the first potential blastocyst transfer. Cumulative rates are additionally planned to be calculated, but as the number of aspirated oocytes is expected to be the same in both treatment groups due to gonadotropin dosing being decided upon independently of allocated treatment group, the effect of the freeze all strategy on the results of the first transfer may be diluted with the inclusion of additional FET´s.

Futhermore, the primary endpoint has been clarified in the manuscript as well where applicable: (P2 L43-45; P5 L117-119; P10 L264-265; P11 L268-269) + (Primary objective, P5, L122-123): Ongoing pregnancy rate per first blastocyst transfer is also considered as a primary aim of the study addressing possible differences in endometrial receptivity between the two groups.

3) Some minor point are furthermore:

a. When the study is completed in May 2018 will that be when results are known for the primary endpoint?

Thank you for this comment. The last patients are expected to be included in the study by the end of May 2018. Subsequently, the primary endpoint, ongoing pregnancy rate per first blastocyst transfer (confirmed at ultrasound scan in gestational week 8), will be known approximately 4 months after the end of inclusion for the patients allocated to the freeze-all and transfer later group. This has been emphasized in the manuscript (Study design: P6, L145-148, clean version):

(Study design: P6, L145-148, clean version): Patient enrolment started in May 2016 and the last patients are expected to be included in the study in May 2018 with the primary outcome measure, ongoing pregnancy rate, being known for these patients approximately four months later for the patients allocated to the freeze-all group.

b. Why only one Bologna poor responder criterion for exclusion and not also for example one of the other criteria such as > 38 yrs or previous poor response?

Thank you for commenting on this. The bologna criteria were not initially used as criterion for exclusion in the study as the study does not aim to categorically exclude poor responders. We have intendedly aimed at broadening the inclusion criteria to test the concept of a freeze-all strategy in a broad population of patients not only limiting the results to a highly selected group of patients. The AMH cut-off value at 6.28 pmol/L (Roche Elecsys assay) was chosen to have a fair chance of the patient ending up with at least one blastocyst to transfer/freeze on day 5 after aspiration.

c. Why is the doctor with his/her selected starting dose (and what contributed to the choice?) part of the treatment variability? I assume that each clinic has its own protocol and why was that not taken as starting point? How was this dose selection put into the randomization program exactly?

In all the participating clinics the individual dosing approach is the standard treatment protocol. For this reason we have chosen to apply this approach in the study as well, allowing the treating doctor at the time of inclusion to choose both treatment drug and starting dose as appropriate. If choosing a fixed dose regimen we would expect to see a larger population of patients either being hyperstimulated or not developing a satisfactory number of follicles given the study inclusion criteria allowing a rather broad population of patients to be included. By choosing the individual dosing regimen we aim to mimic the general practice in the clinics and enable fewer cancellations as the chosen treatment dose has been individually assessed for each patient concerned.

In the computerized randomisation program there is a separate box for the starting dose of FSH to be entered. Entering the FSH starting dose is necessary to be allowed to complete the randomisation program making sure that this has been determined prior to the allocation to one of the two treatment groups and starting FSH dose is therefore not part of the minimization algorithm.

d. I miss MZ twinning rate as secondary endpoint.

Thank you for this comment. As the study is unfortunately underpowered to estimate MZ twinning rate this was not included as a secondary endpoint. The variable is however still incorporated into the collected data and is documented at the ultrasound scan at gestational week 8 and was always meant to be reported in the final paper.

e. What instructions do patients get with regard to intercourse around time of frozen/thawed ET?

No restrictions are given with regard to intercourse around the time of frozen thawed embryo transfer in accordance with general practice in the clinics. We are aware of the fact that there is always a small chance of spontaneous conception, but this may be equally present in both treatment groups. This is a relevant point to be discussed in the final paper.

f. Beta-hCG is not the type of hCG we measure in pregnant women. It is the intact heterodimer of hCG.

Thank you for commenting on this, we have corrected this in the manuscript (Treatment arms and interventions: P9 L224-226, clean version). The hCG determined in plasma is the sum of human chorion gonadotropin plus the hCG β-subunit.

g. Page 9 line 22: Women as well as male partners will answer a questionnaire. Are couples included with a female partner?

Thank you for commenting on this. Women can be included in the study whether they are single, with a female or with a male partner. Questionnaires are however only given to the women undergoing ovarian stimulation and the male partners. We acknowledge that QOL in the female partner population is also an interesting parameter to investigate, but as these couples are a minority in the clinics and the number of answered questionnaires from this we group would be sparse, we have chosen not to include a separate questionnaire for the female partners.

I suggest to address the points made extensively in the discussion.

Reviewer 2: K Coetzee, Antalya IVF, Turkey

Thanks to Reviewer 2 for the relevant comments.

Comments for authors:
A “Freeze-all” versus a “Fresh embryo transfer”-strategy in assisted reproductive technology (ART): Study protocol for a multicentre randomized controlled trial

Stormlund et al BMJ open
This study aims to compare ongoing pregnancy and live birth rates between a freeze-all strategy with GnRH agonist triggering versus hCG trigger and fresh embryo transfer in a multicentre randomized controlled trial.

The authors need to consistently describe the study as the comparison of two strategies, one that includes an hCG trigger and fresh ET and the other a GnRH trigger and FET. Therefore the title of the study may be misleading.

Thank you for this comment. The title has been modified in accordance with this and added to the manuscript (Title: P1, L 3-6, clean version)

(Title: P1, L 3-6, clean version): Comparison of a “freeze all” strategy including GnRH agonist trigger versus a “fresh transfer” strategy including hCG trigger in assisted reproductive technology (ART) – A study protocol for a randomised controlled trial

Although the study as described in the title and objectives only compares two IVF strategies in terms of ongoing pregnancy, it is obvious that the study includes multiple sub-studies. The authors may need to more deliberately express this intention.
Thank you for this comment. The following has been added to the manuscript (Outcome measurements (primary and secondary): P11, L 271-279, clean version):

(Outcome measurements (primary and secondary): P11, L 271-279, clean version): Other endpoints explored in the study contribute to the assessment of other relevant aspects of the freeze-all strategy including ongoing pregnancy rates per transfer, per started stimulation and per oocyte pick-up (percentage of participants with an ultrasound confirmation of foetal heart beat at gestational age 7-8) as well as live birth rate and cumulative live birth rates (percentage of participants with 1 live born neonate after 1 year of follow-up). The study furthermore aims to document the prevalence of OHSS assessed by the number of patients admitted to hospital under this diagnosis and the number of patients having ascites puncture. In addition, it is planned to evaluate pregnancy related complications as well as neonatal outcomes in both groups. For complete overview of all secondary endpoint measures see Table 3.

Because this study in its entirety (full duration) includes the collection of live birth outcomes and beyond, it seems unusual that the primary objective has been written as follows:

The primary endpoint is to compare ongoing pregnancy rates in the two treatment groups after the first single blastocyst transfer.

Thank you for commenting on this. The question is similar to the first reviewer´s question 2, please see the answer above.

The authors need to fully describe how the following 'fresh embryo transfer' subgroup will be analyzed;
If > 18 follicles with a diameter >11 mm are observed, GnRH agonist triggering should be used and all blastocysts frozen (Papanikolaou et al., 2011).

Thank you for this very relevant comment. As the primary endpoint is ongoing pregnancy rate per randomised patient after the first potential blastocyst transfer, the patients allocated to the fresh embryo transfer group who receive GnRH agonist triggering and have all blastocysts frozen due to the severe risk of developing OHSS will still be analysed as part of the fresh transfer strategy group after the intention-to-treat principle. Their first blastocyst transfer will be in the first FET cycle and ongoing pregnancies from such transfers will be included in the numerator together with ongoing pregnancies from patients with first blastocyst transfer in the fresh cycle. It is very important that these patients remain in the allocated group as we would otherwise get an uneven distribution of the patients with more high responders and good prognosis patients ending up in the freeze-all group. We have tried to clarify this in the manuscript: (P13, L289-296, clean version):

(P13, L289-296, clean version):
Patients in fresh embryo transfer group with GnRH agonist triggering

Patients allocated to the fresh transfer group who end up receiving GnRH agonist trigger and vitrification of all blastocysts due to risk of OHSS (>18 follicles with a diameter >11 mm on trigger day) will still be analysed as part of the fresh transfer group according to the intention-to-treat principle. Their first blastocyst transfer will derive from their first FET cycle and ongoing pregnancies from these first transfers will be included in the numerator together with ongoing pregnancies derived from the majority of patients with first blastocyst transfer in the fresh cycle. The denominator will be all randomised patients.

In multinational multicentre studies, heterogeneity is always a concern and a significant source of bias. Even though the randomization will be performed according to trial site, the study can only be strengthened if the outcomes from each centre prior to the start of the study are shown to be consistent.

This is a very relevant comment as heterogeneity is always a concern in multicentre RCT´s. However we only included larger clinics in Denmark and Sweden thus the heterogeneity should be limited as the background populations are rather similar. Furthemore, trial site was included in the randomisation program in line with a range of other background characteristics. Therefore we consider the risk of major heterogeneity unlikely.

VERSION 2 – REVIEW

REVIEWER	Cornelis B (Nils) Lambalk VU University MedicalCenter, Amsterdam
REVIEW RETURNED	11-Apr-2017

GENERAL COMMENTS	The authors have satisfactory addressed the raised issues

REVIEWER	Kevin Coetzee Antalya IVF, Antalya, Turkey
REVIEW RETURNED	10-Apr-2017

GENERAL COMMENTS	**1. A "Freeze-all" versus a "Fresh embryo transfer"-strategy in assisted reproductive technology (ART): Study protocol for a multicentre randomised controlled trial** **2. Comparison of a "freeze all" strategy including GnRH agonist trigger versus a "fresh transfer" strategy including hCG trigger in assisted reproductive technology (ART) – A study protocol for a randomised controlled trial** Stormlund et al The present manuscript now represents a good directive and description of the anticipated study: to answer the question whether the conventional mode of IVF treatment needs to be changed to improve the overall outcomes of IVF. Choosing strategy or intervention is always going to be a matter of complication and compromise, the chosen route of the authors is an appropriate one. The following are points of refinement or interest.

	1. The term one complete cycle may in the context be ambiguous. 126 1. To assess cumulative live birth rates after *one complete cycle* including consecutive single blastocyst 2. Why has the "potential" day of transfer been described as, "performed six or seven days after the hCG injection", if seven days is equivalent to that used for fresh blastocyst transfers. In FET cycles a single injection of 250 ug hCG is administered, when the leading follicle is > 17 mm. Blastocyst transfer is performed six or seven days after the hCG injection. No luteal phase support is given. 3. Because the primary outcome measure (live birth vs clinical or ongoing pregnancy) is critical to the success of the study, I wonder whether the definition proposed by the authors would be most appropriate. Because technically the one proposed is that of a clinical pregnancy, with an ongoing pregnancy most often defined as a pregnancy developing beyond 7-8 weeks of gestation. 120 Ongoing pregnancy rate is defined as an intrauterine pregnancy with a foetal heart beat at transvaginal ultrasound in gestational week 7-8. 4. If patient psychology (i.e., stress and anxiety) plays a role in reproductive outcome I wonder how patients may react after this decision point. When ovulation trigger is decided, the result of the randomisation is disclosed to both doctors and patients and ovulation and oocyte maturation is triggered with a GnRH agonist trigger injection (0.5 mg Buserelin) in the freeze-all group or a single injection of 250 µg of hCG in the fresh embryo transfer group.

VERSION 2 – AUTHOR RESPONSE

Reviewer 2: K Coetzee, Antalya IVF, Turkey

Thank you to the reviewer for the relevant comments.

1. A “Freeze-all” versus a “Fresh embryo transfer”-strategy in assisted reproductive technology (ART): Study protocol for a multicentre randomised controlled trial

2. Comparison of a “freeze all” strategy including GnRH agonist trigger versus a “fresh transfer” strategy including hCG trigger in assisted reproductive technology (ART) – A study protocol for a randomised controlled trial

Stormlund et al
The present manuscript now represents a good directive and description of the anticipated study: to answer the question whether the conventional mode of IVF treatment needs to be changed to

improve the overall outcomes of IVF.

Choosing strategy or intervention is always going to be a matter of complication and compromise, the chosen route of the authors is an appropriate one.

The following are points of refinement or interest.

1. The term one complete cycle may in the context be ambiguous.
126 1. To assess cumulative live birth rates after one complete cycle including consecutive single blastocyst

Thank you very much for commenting on this. We have now added the word “treatment” in the sentence to clarify the term of “one complete treatment cycle” (Secondary objectives, P5, L 126-128)

(Secondary objectives, P5, L 126-128): To asses cumulative live birth rates after one complete treatment cycle including consecutive single blastocyst transfers of all embryos deriving from that oocyte retrieval (fresh and frozen) in the two study groups

2. Why has the "potential" day of transfer been described as, "performed six or seven days after the hCG injection", if seven days is equivalent to that used for fresh blastocyst transfers.
In FET cycles a single injection of 250 ug hCG is administered, when the leading follicle is > 17 mm. Blastocyst transfer is performed six or seven days after the hCG injection. No luteal phase support is given.

Thank you for commenting on this. We completely agree that in the optimal setting the embryo transfer would be done on day 7 in FET cycles as well, however, as the study is done in a multicenter setting, we have had to adjust the protocol to accommodate all participating clinics, and as some of these are unfortunately closed on Sundays, it has been necessary to introduce the possibility of embryo transfer on day six as well in the FET cycles. The day of embryo transfer (day six or seven) will be noted and documented in the database.

3. Because the primary outcome measure (live birth vs clinical or ongoing pregnancy) is critical to the success of the study, I wonder whether the definition proposed by the authors would be most appropriate. Because technically the one proposed is that of a clinical pregnancy, with an ongoing pregnancy most often defined as a pregnancy developing beyond 7-8 weeks of gestation.
120 Ongoing pregnancy rate is defined as an intrauterine pregnancy with a foetal heart beat at transvaginal ultrasound in gestational week 7-8.

Thank you very much for this relevant comment. We have chosen to use this definition in the study as this is the time where all patients treated at the participating clinics are routinely having an ultrasound scan to see if they have a viable pregnancy. According to the WHO definition, a clinical pregnancy is defined as “a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive signs of pregnancy”, whereas an ongoing pregnancy is defined as “a clinical pregnancy of at least 10 weeks of gestational age with a foetal heartbeat”. We completely agree that the optimal endpoint measure of ongoing pregnancy would be that proposed by the WHO, but as the patients are only scanned at gestational week 7-9 at the clinics, and the measure used at this timepoint to define the ongoing pregnancy is an ultrasonographically verified living foetus and not just a gestational sac or other signs of pregnancy, we think that the chosen definition is a reasonable approximation.

4. If patient psychology (i.e., stress and anxiety) plays a role in reproductive outcome I wonder how patients may react after this decision point.

When ovulation trigger is decided, the result of the randomisation is disclosed to both doctors and patients and ovulation and oocyte maturation is triggered with a GnRH agonist trigger injection (0.5 mg Buserelin) in the freeze-all group or a single injection of 250 µg of hCG in the fresh embryo transfer group.

The participants, both men and women, answer quality of life related questionnaires two times during the study period. The first questionnaire is answered 4 days after oocyte pick up and the second one 16 days after oocyte pick-up. In these questionnaries we explore thoughts, feelings, expectations and concerns regarding the treatment process and in that context possible strain in relation to the waiting period for the participants allocated to the freeze-all study arm should also be exposed. Whether the results from the questionnaires can be linked to reproductive outcomes would be an interesting aspect to look into.

VERSION 3 – REVIEW

REVIEWER	Kevin Coetzee Antalya IVF, Antalya, Turkey
REVIEW RETURNED	16-May-2017

GENERAL COMMENTS	The study protocol is now ready for publication.