Author’s response to reviews

Title: D6 blastocyst transfer on day 6 in frozen-thawed cycles should be avoided: a retrospective cohort study

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Tovah Honor Aronin
Editor
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Dear Editor,

Thank you for the opportunity to resubmit our revised manuscript. We thank you and the reviewers for your constructive suggestions and comments. Our point-by-point response to the reviewers’ comments are below, and the revisions are indicated as text highlighted in red in the revised manuscript.

We hope that the revised manuscript is now acceptable for publication. We look forward to hearing from you soon.

Best wishes,

Yours sincerely,

Huiling Xu, on behalf of
Dr Beihong Zheng. Chief Physician
Reproductive Medicine Center, Fujian Maternity and Child Health Hospital
We express our sincere thanks to the reviewers for the constructive and positive comments.

Replies to Reviewer 1

1) The title should be shortened and focused.
Answer: Thank you for the suggestion. The title “D6 blastocysts transfer on day 6 in frozen-thawed cycles should be avoided, and early miscarriage rate of D5 blastocysts is lower than D6 blastocysts.” has been revised as “D6 blastocyst transfer on day 6 in frozen-thawed cycles should be avoided: a retrospective cohort study”.

2) Patients:
The authors stated that "D6 blastocysts are transferred on day 6 in natural FET cycles to avoid transfer on weekend. While in some other in vitro fertilization (IVF) clinics, including our center, D6 blastocysts were planned to be transferred on day 6 according to the synchronization between the endometrium and the embryos" in the Introduction section. They may also declare how the time of D6 embryo transfer was planned in their dataset in the Materials and Methods section.
The authors suggested a "day 6 according to the synchronization between the endometrium and the embryos". How did they determine the synchronization and the timing? It should be detailed in the Materials and Methods.
In Table 1, data shown that the proportion of NC/HRT cycles was comparable between day6 on day 5 and day 6 on day 6 group. How many day 6 on day 6 cycles are due to logistic reason and many are due to endometrial synchronization?
Answer: Thank you for your suggestion. We apologize for the unclear description. In our center, we believed that D3 embryos should be transfer on day 3, D5 blastocysts on day 5, and therefore D6 blastocysts should be synchronized with endometrium on day 6. We did not perform endometrial histological examinations but logistically considered that D6 blastocysts would be synchronized with the endometrium on day 6. However, we were wrong based on the results of the study.
We described this as “logistic” in the revised manuscript in accordance with your suggestion.

3) Statistics:
The authors used multivariate analyses in the study. The selection of the covariates should be detailed in the Materials and Methods section.
Because the pregnancy rates are related to many factors and day 6 FET is related to several biases, I suggest the authors consider additional and biases.
For instance, the D5 embryos were transfer in priority in the present study. It may suggest that patients with D6 FET have more previous failures and therefore have poor prognosis. Important predictors for pregnancy such as age and endometrial thickness should also be included in the multivariate model, regardless they are significant or not in the univariate analysis.
In addition, the authors considered the "Day of blastocyst formation" and "Timing for blastocyst transfer" as independent factors. However, timing and day of blastulation are closely related in the present study. D6 transfer was only for day 6 embryos. It may be problematic to consider these two factors independent. One may replace them with a three-level parameter (eg. day 6 on day6, day 6 on day5 and day 5 on day 5).

Answer: Thank you very much for your constructive and valuable suggestions. In the revised manuscript, we considered possible covariates including previous failures, age and endometrial thickness as confounders, and found that the maternal age and endometrial thickness were significantly associated with CPR.

We would like to share our opinion about the reason we considered "Day of blastocyst formation" and "Timing for blastocyst transfer" as independent factors. When we replaced them with a three-level parameter, the OR was 3.556 (95 % CI:2.804-4.511, P=0.000) and 2.041 (95 % CI:1.316-3.166, P=0.001), higher than 2.041 and 1.742 reported in the original manuscript. However, we believe that it is more meaningful to consider them separately for guiding the clinic practice. The results may help the doctors to decide the type of blastocyst, and the time of the blastocysts transfer should be a priority. I hope you could agree with us.

4) Tables:
I suggest the authors integrate the CPR/IR into the Tables.
Answer: The pregnancy outcomes have been integrated into the Tables, in accordance with your suggestion.

Replies to Reviewer 2

1) Although the primary language of the authors is not English, there are multiple errors in grammar and syntax which should be corrected.
Answer: We have sought professional help in revising this manuscript, and the grammar and syntax errors have been corrected.

2) There is clearly a significant difference in cycle numbers between the groups. The authors do not describe how patients undergoing day 6 blastocyst transfer were triaged to one approach or the other. This clearly represents the potential for selection bias.
Answer: In our center, D6 blastocysts used to be planned to be transferred on day 6. However, some of the doctors changed their opinions and believed D6 blastocysts should be transferred on day 5, so if only it was their turn to decide the transfer time for the patients, they chose day 5, regardless the blastocyst quality or any other factors. Meanwhile, we used to take turns on duty. I think it may be considered as kind of randomization, although not strictly.

3) The authors do not describe differences in embryo quality between the groups.
Answer: Thank you very much for your constructive and valuable suggestions. We confirmed the issue of embryo quality with our embryologist again and have added the data to the tables. The data were analyzed using the multivariate logistic regression analysis. We found that we had used the wrong description about the blastocyst quality erroneously in the original manuscript. We have corrected it in the Materials and Methods section of the revised manuscript. Thanks to you, we have found that the proportion of good quality blastocysts in the D6-on-D5 group and
D6-on-D6 group was comparable; however, the proportion of good quality blastocysts in the D5 group was significantly higher than that in the D6-on-D5 group (87.6% VS 58.3%, P=0.000).

4) Given the fact that there are multiple studies showing that transfers after 6 days of progesterone are less successful, why would the authors consider doing so in the first place? There are indeed some ethical considerations in this regard that should be addressed.

Answer: Thank you for your question. At the beginning of blastocyst transfer in our center, there were no published studies to refer to. We believed that the D3 embryos should be transferred on day 3, D5 blastocysts on day 5; and thus, D6 blastocysts should be synchronized with endometrium on day 6. However, we were wrong based on the results of the manuscript. Nowadays, we transfer the D6 blastocysts on day 5 in FET cycles and as I mentioned in the discussion “Although a properly designed randomized study would be needed to obtain conclusive evidence, the present results with a highly clinically relevant reduction in CPR may render it ethically difficult to accept strict randomization between day 5 and day 6 transfer for D6 blastocysts.”

“Several studies provided evidence that blastocysts transfers in fresh cycles should be performed on day 5, and blastocysts transfer on day 6 in fresh cycles should be avoided [1-3]. However, there is no powerful evidence about the suitable timing to transfer D6 blastocysts in FET cycles.”

We believe that “Our analysis is the first known large study to investigate the effect of timing to transfer D6 blastocysts in FET cycles on clinical outcomes.” We will be glad that D6 blastocysts transfer on day 6 in FET cycles could be absolutely avoided if my revised manuscript could be accepted for publication with your permission and the results of this study could be aware by the doctors.

5) Line 65 - The authors state that one reason that day 6 blastocysts were transferred on day 6 is to avoid a weekend transfer. The authors should explain why they would compromise patient for convenience in light of the fact that outcomes were known to be poorer in this group.

Answer: Thank you for your question. Before we completed the data analysis of this manuscript, most doctors in our center believed that day 6 blastocysts transfer on day 6 would be better for the patients. Meanwhile, we work even on weekends to provide the best possible care to patients. Hence, this would not happen in our center. Nevertheless, we believe that this might happen in some IVF clinics where the doctors do not work on weekends and they are unaware that “The clinical pregnancy rate(CPR) and implantation rate(IR) were significantly higher in the D6-on-D5 group than in the D6-on-D6 group (55.3% vs. 37.3%, 44.8% vs. 32.6%, P<&lt;0.01)” based on the results of our study.

We wrote this reason based on a reviewer’s comment “this manuscript does not provide any new information nor would expand our horizon in the matter regarding vitrification of human blastocysts. It is standard of care today, that vitrified human blastocysts regardless of their stage of development get warmed and transferred on day 5 of progesterone supplementation.” Yet, we believe that the results will help doctors make their decision in the clinic, especially after we found that HRT cycles exhibited a higher EMR than natural cycles (OR:1.959, 95% CI:1.305-2.939, P=0.001) in the revised manuscript.

We appreciate your comment “This is an interesting paper adds to information that is already present in the literature.” Thank you very much for the opportunity to resubmit our revised manuscript and we hope that the revised manuscript is now acceptable for publication.
6) The authors did not compare outcomes with natural versus prepared cycle, stating that they did not do so because several studies showed no difference. Since these studies were not performed in the authors' institution, it is why this would be a valid reason. It would be critical to determine if the nature of endometrial preparation represents a confounding variable and therefore, further evaluation of patients who had a prepared versus natural cycle embryo transfer would be critical if endometrial preparation is indeed an issue.

Answer: Thank you very much for your constructive and valuable suggestions. We considered endometrial preparation as a confounder in the multivariate logistic regression analysis, and found that although there was no difference observed in the CPR, HRT cycles exhibited a higher EMR than natural cycles (OR:1.959, 95% CI:1.305-2.939, P=0.001). The results are beyond our expectation and we wish to extend our thanks to you.

7) Are there differences in embryo quality between the groups?

Answer: Thank you for your suggestion. We have added the data and found that the proportion of good quality blastocysts in D6-on-D5 group and D6-on-D6 group were comparable; however, the proportion of good quality blastocysts in the D5 group was significantly higher than that in the D6-on-D5 group (87.6% vs. 58.3%, P=0.000).

References