

## Peer Review File

Article information: <http://dx.doi.org/10.21037/atm-20-3230>

### **Response to Reviewer #A Comments**

**Comments:** This manuscript represents an attempt to determine the impact of thin endometrial lining on pregnancy outcome in fresh and frozen embryo transfer cycles in IVF.

The authors defined thin endometrium as  $<7.5$  mm on the day of hCG triggering. Out of a total of 1664 patients with thin endometrium (1037 fresh embryo transfers and 627 frozen embryo transfers) the authors used propensity score matching (PSM) to select 243 patients in each fresh and frozen embryo transfer group for comparison. They expected that PSM for several IVF variables would reduce the potential bias resulting from the retrospective nature of the study. The results of this research study showed a significantly increased live birth rate, clinical pregnancy rate (CPR) and implantation rate with frozen embryo transfers compared to fresh embryo transfers in women with thin endometrium ( $<7.5$  mm).

**Reply:** Thank you for putting time and effort into reviewing the previous version of the manuscript. The suggestions have enabled us to improve our work.

**Comment 1:** I believe that the definition of thin endometrium in this study is problematic. The 2014 meta-analysis by Kasius et al reviewed 10,724 fresh embryo transfer cycles from 23 papers and showed that clinical pregnancy rate was 23.3% if endometrial thickness was 7 mm or less and 48.1% if  $>7$ mm (OR 0.42, 95% CI 0.27-0.67). The largest study to date by Liu et al (2018) examined 24,363 fresh embryo transfer cycles and 20,114 frozen embryo transfer cycles. They demonstrated a significant decrease in CPR and live birth rate for each millimeter decline in endometrial thickness below 7 mm in frozen embryo transfers and for each

millimeter decline in endometrial thickness below 8 mm for fresh embryo transfers. Therefore, selection of 7.5 mm in the present study may inherently bias the results against fresh embryo transfers. A better definition of thin endometrium would be  $<7$  mm. Alternatively, a definition of  $<7$  mm in frozen embryo transfers and  $<8$  mm in fresh embryo transfers may be more appropriate. I would suggest reanalysis of the data using both definitions, i.e.  $<7$  mm for both fresh and frozen, or  $<8$  mm for fresh and  $<7$  mm for frozen embryo transfers.

**Reply 1:** Special thanks to you for your good comments. We redefined thin endometrium to be  $\leq 7$  mm on HCG trigger day as Reviewer suggested and Kasius et al(1) mentioned in the literature. In total, we identified 1110 women who met the study criteria, including 632 women who had a fresh embryo transfer cycle and 478 women who had a frozen embryo transfer cycle. After PSM, 173 patients were included in two groups respectively. According to the Reviewer's suggestion, we reanalysis the data using the new definition of thin endometrium (EMT  $\leq 7$  mm on HCG trigger day). We remade the table and the conclusion is the same as before.

**Changes in the text:** Page 4, Line 76-78 & Page 8-10, Line 158-191.

**Comment 2:** It is unclear from the methodology if the thin endometrium in this study was diagnosed only on the day of hCG trigger in fresh cycles or was also defined as measured at the end of the follicular phase in natural cycle FETs or at the end of the estrogen phase in HRT FET cycles. It is important to know if the endometrial thickness was thin before starting the luteal phase or progesterone support in the FET cycles. This information is required in the materials and methods section.

**Reply 2:** Thank the Reviewer for this precious comment. In our study, thin endometrium was diagnosed as  $\leq 7$  mm on hCG trigger day according to the Reviewer's suggestion. Facing this group of people, clinicians may often be faced with the dilemma of whether they should continue the fresh IVF cycle or cryopreserve the embryos to wait for a better chance of pregnancy during the subsequent frozen ET cycle. We strongly agree with the Reviewer that it is important to know if the endometrial thickness was thin before starting the luteal phase or progesterone support in the FET cycles. Endometrial thickness (EMT) was also measured at the end of the follicular phase in natural cycle FETs or at the end of the estrogen phase in HRT FET cycles. We compared the EMT on HCG day and before embryo transfer in FET group. The results of our study suggested that for women who had a thin endometrium, the EMT increased in frozen ET cycles compared with their EMT on HCG trigger day (see Table II). As Reviewer suggested, we have added this in the materials and methods section. Thank you very much.

**Changes in the text:** Page 6-7, Line 121-128.

**Comment 3:** How were patients selected for fresh embryo transfer vs freeze all and frozen embryo transfer? It appears that BAF counts were the same in the two groups. Were the number of oocytes retrieved also the same in both fresh and frozen embryo transfer groups? Were freeze all cycles used in women who overstimulated to reduce the risk of OHSS or were fresh transfer or freeze all cycles selected at physician discretion? Please discuss.

**Reply 3:** Special thanks to you for your good comments. In general, indications for the “freeze all” strategy included moderate to severe ovarian hyperstimulation syndrome (OHSS), a high

risk of moderate to severe OHSS, a thin endometrium, the presence of progesterone elevation, and other factors affecting embryo implantation. Specifically, some patients participated in our hospital's randomized, controlled trial (2, 3) and were randomly assigned to the Frozen-Embryo group. Our study intends to analyze the differences of pregnancy outcomes between fresh and frozen-thaw embryo transfer (ET) for patients with thin endometrium. We screened all thin endometrial patient (EMT  $\leq$ 7 mm on HCG trigger day) who underwent IVF/ICSI-ET treatment between January 2013 and December 2017 in the unit. Patients who had a fresh ET were included in the Fresh-Embryo group, and patients who underwent frozen ET adopted “freeze-all” strategy after oocytes retrieval were included in the Frozen-Embryo group. The study is retrospective, and patients were not randomly assigned. Therefore, we used propensity score matching (PSM) to reduce the potential bias. As Reviewer suggested, we compared the number of oocytes retrieved between the two groups. The number of oocytes retrieved in Frozen-Embryo group was more than that in the Fresh-Embryo group (10.49 $\pm$ 7.76 vs. 7.19 $\pm$ 4.82,  $P < 0.001$ ). After matching, no significant differences were observed in number of oocytes retrieved between the two groups ( $P > 0.05$ ). Baseline characteristics of the two groups of patients we included were balanced after PSM.

Thanks again for your suggestions. We have added the explanation for this in the discussion.

**Changes in the text:** Page 11, Line 222-227 & Page 7-8, Line 140-144 & Page 8, Line 147.

### **Response to Reviewer #B Comments**

**Reply:** Thank you very much. We have revised our manuscript according to your kind suggestion and comments.

**Comment 1:**

Methods: mention the protocols and medications used for COS

more details about embryos freezing

describe more PSM- what was confounding factors to remove the bias?

**Reply 1:** Special thanks to you for your good comments.

Different ovarian stimulation protocols were selected according to the patient's age and ovarian function, including gonadotrophin-releasing hormone (GnRH) agonist long protocols, GnRH agonist short protocols, flexible GnRH antagonist protocols, mild stimulation protocols, and natural cycle protocols, which have been reported in detail previously (4). We have modified our text as advised. (see Page 5, line 94-98)

For women in the Frozen-embryo group, all available embryos are vitrified. Some good-quality embryos were cryopreserved using vitrification on day 2 or day 3, and the other embryos could be vitrified at the cleavage or blastocyst stage. Vitrification was carried out following the Mukaida protocol with cryoloop (5). The embryos were first placed into the basic medium (HEPES-buffered modified hTF medium), and then transferred into the basic medium containing 7.5% (v/v) dimethylsulfoxide (DMSO) and 7.5% (v/v) ethylene glycol (EG; vitrification solution I) for 2 minutes. Finally, the embryos were suspended for 30 seconds in the basic medium containing 15% (v/v) DMSO, 15% (v/v) EG, 10 mg/ml Ficoll 70, and 0.65 mol/l sucrose (vitrification solution II), and were plunged into liquid nitrogen quickly. Warming was carried out in a four-well multidish according to the Mukaida protocol. The embryos were incubated in a basic medium containing 0.33 mol/l sucrose (thawing solution I),

a basic medium containing 0.2 mol/l sucrose (thawing solution II), and a basic medium for 2, 3, and 5 minutes at 37 °C, respectively. (see Page 6, line 103-115)

The study is retrospective, and patients were not randomly assigned. So inevitably there are potential confounding and selection bias could affect the outcomes. Therefore, we used propensity score matching (PSM) to identify Fresh-Embryo group patients who were most identical to the Frozen-Embryo group patients. We assigned propensity scores to the baseline characteristics and embryo transfer variables that potentially affect the pregnancy outcomes. We used a logistic regression model to calculate propensity scores based on female age, body mass index (BMI), duration of infertility, antral follicle count in both (AFC), number of oocytes retrieved, basal FSH, basal LH, type of infertility, proportion of patients with PCOS, EMT on the HCG day, number of embryo transferred, and stage of embryo transferred. These variables mentioned above were listed as baseline characteristics and embryo transfer variables. Twelve independent variables were used to determine the propensity score, thus to remove bias. We have modified our text as advised. (see Page 7-8, line 140-144 & Page 8, line 150-152)

**Comment 2:**

Results: interpret your results- describe the results more- with and without PSM results

**Reply 2:** Thanks for your comment. Before matching, baseline characteristics and embryo transfer variables were unevenly distributed between the two groups of patients. The patients in the Fresh-Embryo group were older ( $34.75 \pm 4.65$  years vs.  $33.57 \pm 5.42$  years,  $P < 0.001$ ) and had higher basal FSH ( $7.95 \pm 3.39$  vs.  $7.32 \pm 3.59$ ,  $P = 0.003$ ). The Fresh-Embryo group patients showed fewer antral follicle count in both ovaries ( $11.29 \pm 8.04$  vs.  $13.92 \pm 10.00$ ,  $P$

<0.001), fewer retrieved oocytes ( $7.19 \pm 4.82$  vs.  $10.49 \pm 7.76$ ,  $P < 0.001$ ), fewer patients with PCOS (9.5% vs. 15.3%,  $P = 0.003$ ), thicker EMT on the hCG day ( $0.66 \pm 0.06$  cm vs.  $0.62 \pm 0.10$  cm,  $P < 0.001$ ), fewer blastocyst embryos (17.2% vs. 85.4%,  $P < 0.001$ ), and fewer single embryo transferred (35.3% vs. 76.6%,  $P < 0.001$ ) than the Frozen-Embryo group patients. After matching, the baseline characteristics and embryo transfer variables of the patients were similar in the two groups, as shown in Table 1. No significant differences were observed in age, infertility duration, body mass index, antral follicle count in both ovaries, number of oocytes retrieved, baseline FSH, baseline LH, the proportion of patients with PCOS, endometrial thickness on HCG trigger day, stage of embryo transferred or number embryo transferred between the two groups ( $P > 0.05$ ). We have made supplements and modifications in the Results. (see Page 9, line 164-177)

**Comment 3:**

Discussion:

studies describing cut off of 7 or 8. why did you choose 7.5 mm? kindly explain

write your opinion for findings of others. why do you think they found this? explain more

**Reply 3:** Thank you for the comment. In 2014, Kasius et al.(1) conducted a systematic review and meta-analysis. The results showed that the clinical pregnancy rate with EMT  $\leq 7$  mm was significantly lower than with EMT  $> 7$  mm. A recent systematic review showed that both clinical pregnancy and live birth rate decreased significantly for each millimeter of endometrial thickness below 8 mm in fresh IVF cycles and below 7 mm in frozen-thaw IVF cycles(6). Endometrial thickness might be associated not only with successful embryo implantation but

also with further fetal development. A recent study based on 864 singletons produced by fresh IVF–ET cycles found that an EMT<7.5 mm was associated with an increased risk of obstetric pregnancy complications(7). Our recent study found that women with EMT  $\leq$ 7.5 mm still had a higher risk of delivering an SGA fetus than women with EMT >12 mm(4).

Reviewer #A suggested that a better definition of thin endometrium would be  $\leq$ 7 mm. We redefined thin endometrium to be  $\leq$ 7 mm on HCG trigger day as Reviewer #A suggested and Kasius et al mentioned in the literature. In total, we identified 1110 women who met the study criteria, including 632 women who had a fresh embryo transfer cycle and 478 women who had a frozen embryo transfer cycle. After PSM, 173 patients were included in two groups respectively. According to the Reviewer's suggestion, we reanalysis the data using the new definition of thin endometrium (EMT  $\leq$ 7 mm on HCG trigger day). We remade the table and the conclusion is the same as before. (see Page 4, Line 76-78 & Page 8-10, Line 158-191)