Does estradiol orally and vaginally administered together impact live birth and neonatal outcomes in artificial frozen-thawed embryo transfer cycles: a retrospective cohort study

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Abstract

Background: Previous studies have demonstrated that newborns from fresh embryo transfer have higher risk of small for gestation (SGA) rate than those from frozen-thawed embryo transfer (FET). It is suggested that supraphysiologic serum estradiol in controlled ovarian stimulation (COS) is one of reasons. Our study aims to investigate whether exogenous estradiol delivered regimens have an impact on live birth rate and neonatal outcomes in hormone replacement (HRT)-FET cycles.

Methods: This was a retrospective study involving patients undergoing their first FET with HRT endometrium preparation followed by the transfer of two cleavage-staged embryos, comparing estradiol administered orally and vaginally (OVE group) versus estradiol administered orally (OE group) from January 2015 to December 2018 at our center. A total of 792 patients fulfilled the criteria, including 228 live birth singletons. The live birth rate was the primary outcome measure. Secondary outcome measures included clinical pregnancy rate, singleton birthweight, large for gestational age (LGA) rate, SGA rate, preterm delivery rate.

Results: Patients in OVE group achieved higher serum estradiol level with more days of estradiol treatment. No difference in live birth (Adjusted OR 1.327; 95%CI 0.982, 1.794, p = 0.066) and clinical pregnancy rate (Adjusted OR 1.278; 95%CI 0.937, 1.743, p = 0.121) was found between OVE and OE groups. Estradiol route did not affect singletons birth weight (β = -30.962, SE = 68.723, p = 0.653), the odds of LGA (Adjusted OR 1.165; 95%CI 0.545, 2.490, p = 0.694), the odds of SGA (Adjusted OR 0.569; 95%CI 0.096, 3.369, p = 0.535) or the preterm delivery (Adjusted OR 0.969; 95%CI 0.292, 3.214, p = 0.959).

Conclusion: Estrogen taken orally and vaginally together did not change live birth rate and singleton neonatal outcomes compared to estrogen taken orally, but was accompanied with relative higher serum E2 level and potential maternal undesirable risks.

Background

Vitrification technology has played an instrumental role in the implementation of fertility preservation, single embryo transfer and freeze-all cycles and helped avoid ovarian hyperstimulation syndrome (OHSS) in assisted reproductive technology (ART). During frozen-thawed embryo transfer (FET), though no endometrial preparation protocol superior to others is found, hormone replacement treatment (HRT) is preferred by many centers due to the controlled exposure to exogenous steroids and adjustment of the timing of transfer. In HRT endometrial prepared FET cycles, estradiol and progesterone are sequentially administered to synchronize the window of embryo implantation with embryo transfer. Ovulation was hampered due to the continued estrogen administration during the initial estrogen-only phase. Daily progesterone started before the scheduled embryo transfer. Adequate estradiol priming induced endometrial cells proliferation and appropriate progesterone receptors to develop endometrial receptivity.

The growing number of FET has made researchers to examine various aspects of this treatment strategy. Epidemiologic data has indicated that FETs are associated with increased risk of large and post-

date babies and adverse perinatal outcomes like pregnancy related hypertension, postpartum hemorrhage, compared to fresh transfer[1-6]. The increased risk of preterm labor and small for gestational age (SGA) babies was found in fresh-transfer ART and was fundamentally decreased in FET. The reasons behind that varied. Though some reports suggested embryo cryopreservation altered epigenetics regulation inducing abnormal placentation and fetal growth, more studies advised the elevated level of estradiol in fresh IVF cycles impaired trophoblastic invasion and led to the smaller birthweight and shorter gestational weeks[7-12]. The negative effect of supraphysiologic E2 milieu on implantation and placentation was believed eliminated by the subsequent transfer of frozen-thawed embryos in the relatively steady endocrine environment. However, the duration and dose of estradiol administration in HRT-FET cycles intervened with the clinical and neonatal outcomes. Sekhon et al. have indicated that duration of estradiol supplementation before progesterone in artificial cycles did not impact frozen blastocyst transfer outcome, but inversely correlated with gestational age[13]. Constant dose or increasing dose of estrogen in HRT for endometrium preparation though didn't change live birth rate[14], but excessive duration of estradiol priming which was more than 28 days induced slacked pregnancy rate and increased miscarriage rates in FET[15].

The oral route of estradiol supplement is simple and well-tolerated, and all early protocol used oral estrogen. The first-pass metabolism can be bypassed by utilizing parenteral routes of estradiol administration. Estrogen administered vaginally was firstly reported in 1988[16]. The alternative vaginal rings allowed a steady release of estrogen into the bloodstream. Estrogen administered vaginally seemed to cause higher circulating E2 level and even higher in endometrial tissue than estrogen administered orally[17]. The objective of the current retrospective study was to investigate whether estrogen administered orally and vaginally together made a difference to the live birth rate and singletons birthweight and gestational weeks compared to estrogen administered orally. This model was chosen to specifically focus on the effect of higher E2 priming induced by estradiol administered vaginally on FET clinical and neonatal outcomes.

Methods

Study design and patient population

This is a retrospective study which was undertaken at the assisted reproduction medicine department of Shanghai General Hospital affiliated to Shanghai Jiao Tong University School of Medicine. It involved 1005 women who had undergone their first FET from January 2015 to January 2018. Data was extracted from the database in our center. Patients inclusion criteria was maternal age < 48, two Day 2 or Day 3 cleavage-staged embryos transfer following HRT endometrium preparation. The following patients were excluded from the analysis: (1) Patients with cryopreserved oocytes or donor oocytes. (2) Patients who had prior attempts at conception via IVF and FET. (3) Patients who were in HRT treatment with Sidenafil or growth hormone to improve endometrium development. (4) Patients with endometrium thickness less than 6mm at progesterone starting day. (5) Patients with endometrial polyps, submucosal myomas, or endometrium separation. (6) Patients with history of multiple induced abortions (≥4 times), diagnosis of

uterine adhesions, uterine malformation like Mullerian anomalies, bicornuate uterus, complete septate uterus, dimetra etc. (7) Patients with estrogen administration beyond the doctor's order. (8) Patients with duration of estradiol administration > 28 days. Of the examined cases only 792 women and 228 live birth singletons were included in this study (Figure 1). Institutional review board and ethics committee of Shanghai General Hospital approval was obtained (2020KY016).

IVF and Laboratory protocols

Ovarian stimulation, oocyte retrieval and fertilization procedures have been previously presented[18]. As for IVF, oocytes were inseminated with human tubal fluid with 10% serum substitute supplement and around 300000 progressively motile spermatozoa. As for ICSI, oocytes were laid in the fertilization medium immediately after microinjection. Fertilization was evaluated 18 hours post fertilization procedures. Embryos were cultured in early cleavage medium before Day3 and in multiblast medium afterwards. All embryos were cultured in incubator at 37°C, under 6% CO_2 and 5% O_2. Embryo development was evaluated on Day 2, Day 3, Day 5 and Day 6 according to the consensus[19]. Day 2 cleavage-stage embryos with at least two blastomeres and less than 20% fragmentation were eligible for cryopreservation. Day 3 cleavage-stage embryos with at least 6 cells and less than 20% fragmentation were eligible for cryopreservation. The criteria for good-quality embryo were: 4 to 6 cells with less than 10% fragmentation for Day 2 embryos, 7 to 9 cells with less than 10% fragmentation for Day3 embryos.

Frozen embryo transfer protocols

In a subsequent cycle, patients were administered estrogen and consecutive progesterone for endometrial preparation before FET. All patients started with oral estrogen (estradiol valerate or estradiol Femoston) 6mg per day from the second day of menstruation and continued for one week. Subsequently, some patients continued the same regimen and "OE group" was used for these patients. Other patients were given extra estradiol supplement by vaginal route, estradiol Femoston 2mg per day, combined with the oral usage for another 7-21 days. "OVE group" was used for these patients (Figure 2). The whole estradiol administration duration varied from 10 to 28 days. We performed transvaginal ultrasonagraphy every week to assess patients' endometrium. Vaginally administered estrogen was usually added if the endometrium thickness was less than 6mm. Additionally, it was also depended on physicians' preference. Serum E2, LH and progesterone level were measured at each visit to detect premature ovulation. Once the timing of FET was determined, progesterone intramuscularly or Crinone vaginally was initiated daily. They both combined with oral dydrogesterone 40mg per day and oral estradiol 6mg per day. Intramuscular progesteron 60mg per day or vaginal Crinone 90mg per day was chosen according to patient preference after fully informing the advantages and side-effect of different routes like vaginal itch and discharge or the subcutaneous swell by intramuscular injection. Patients who were undertaken two Day 2 or Day 3 cleavage-staged embryos transferred started the progesterone 2 or 3 days before FET,

respectively. The vitrification and thawing procedures were previously described[20]. Embryo transfer was performed via the same flexible catheter (20G) and transabdominal ultrasonography guidance. After embryo transfer, same daily estrogen and progesterone administration continued until a negative hCG test at the 14th day after transfer. If pregnancy was achieved, hormone administration continued until 12 weeks' gestation.

Outcome measures and definitions

In order to evaluate the impact of orally and vaginally delivered estradiol on clinical outcome, the primary outcome measure was live birth rate. Clinical pregnancy rate, infant birthweight, LGA, SGA, preterm delivery rate were secondary outcome measures. Live birth was defined as a delivery of a viable infant after 28th gestational weeks. Clinical pregnancy was a pregnancy confirmed by the gestational sac or heart beat under ultrasonography. Gestational age was calculated from 14 days before the embryo transfer. Preterm birth was defined as delivery between 28 to 37 gestational weeks. Small for gestational age (SGA) and large for gestational age (LGA) were defined as birthweight <10th and >90th percentile, respectively. Z score was administered to calculate birthweight adjusted for gestational age and newborn gender with the formula: Z score=(c-m)/s, where c is the newborn birthweight, m is the mean birthweight for the same sex and same gestational age in the reference group and s is the standard deviation of the reference group. The reference is the Chinese singletons newborns[21].

Statistical analysis

Patients and live birth singletons demographic baseline, cycle characteristics, clinical and neonatal outcomes were compared by Student's t-test, Mann-Whitney U test, chi-square and Fisher's exact test, as appropriate. Whether binary live birth and clinical pregnancy were affected by the regimen of estradiol was assessed by multivariable logistic regression adjusting for maternal age, BMI, whether duration of estradiol treatment >21 days, whether at least one good quality embryo was transferred, progesterone route, endometrium thickness at progesterone starting day. Multivariable logistic regression was applied to evaluate estradiol regimen's impact on preterm delivery rate, LGA and SGA rate, adjusting for the major covariates mentioned above plus newborn gender. Multiple linear regression was applied to investigate the independent effect of estrogen regimen on singleton birthweight. Adjusted odds ratios (OR) and 95% confidence intervals (95%CI) were reported. All analyses were conducted with SPSS statistics. P value < 0.05 was considered statistically significant.

Results

Clinical outcome

This analysis included 792 women and 228 live birth singletons with the following outcomes: 45.1% clinical pregnancy rate, 36.4% live birth rate (288 live birth including singletons and twins). There were 324 patients who were given oral and vaginal estradiol (OVE group), and 468 patients who were given oral estradiol (OE group). Baseline demographics and characteristics were compared between patients with different estrogen regimens (Table 1). Among the 792 women, no significant difference of maternal age, BMI, fraction of patients with at least one good quality embryo transferred, progesterone route, E2 level at 14th day after embryo transfer and P level at progesterone starting day, was revealed between two groups. There were more patients with estradiol treatment days over 21 days in OVE group than in OE group. Serum E2 level at progesterone starting day in OVE group was significantly higher than that in OE group. Endometrium thickness at progesterone starting day in OVE group was smaller than that in OE group. Meanwhile, endometrium thickness at trigger day in COS of OVE group was also smaller than that of OE group. No significant difference of live birth rate (Crude OR 1.229, 95%CI 0.917, 1.649) and clinical pregnancy rate (Crude OR 1.260, 95%CI 0.948, 1.675) was found between OVE and OE group (Table 2). Controlling for maternal age, BMI, whether estradiol duration was longer than 21 days, whether there was at least one good quality embryo transferred, progesterone route, endometrium thickness at progesterone starting day, estradiol administered regimen did not modify the odds of achieving live birth (Adjusted OR 1.327, 95%CI 0.982, 1.794, p = 0.066) or clinical pregnancy (Adjusted OR 1.278, 95%CI 0.937, 1.743, p = 0.121) (Table 2). Endometrium thickness at progesterone starting day positively influenced the clinical pregnancy (Adjusted 1.184, 95%CI 1.026, 1.365, p = 0.021) but with no impact on live birth rate (Adjusted OR 1.119, 95%CI 0.968, 1.295, p = 0.130) (Supplementary Figure 1 & 2). Maternal age and at least one good quality embryo transferred were the independent factors positively correlated to the live birth rate and clinical pregnancy rate (Supplementary Figure 1&2).

Neonatal outcome

To further explore the estrogen regimen impact on birthweight and gestational age, a cohort of 228 live birth singletons from 792 patients was further investigated. The singletons were divided in two groups which were OVE group, and OE group. Baseline demographic and cycle characteristics were presented in Table 3. Comparison between the two groups did not reveal any significant difference for maternal age, BMI, whether there was at least one good quality embryo transferred or progesterone route. OVE group from 228 singletons cohort had more percentage of patients with longer days of estradiol treatment, thinner endometrium and higher serum E2 level at progesterone starting day than OE group, as the same with the 792 cohort (Table 3).

Neonatal outcomes stratified by the regimen of estradiol administered were also presented in Table 3. Preterm delivery rate, mean birthweight and Z-scores were not different across two groups (Table 3). Given that gestational age at delivery is an important determinant for newborn birthweight, a subgroup analysis was conducted stratified by term delivery. The analysis of preterm delivery singletons was not performed because the number in this category was too small. As shown in Table 3, among the term singletons, newborn gender, mean birthweight, LGA rate and SGA rate were not different across the two groups.

In multivariate analyses (Table 4), the risk of preterm delivery (Adjusted OR 0.969, 95%CI 0.292, 3.214, p = 0.959) and the risk of LGA (Adjusted OR 1.165, 95%CI 0.545, 2.490, p = 0.694), SGA in term delivery (Adjusted OR 0.569, 95%CI 0.096, 3.369, p = 0.535) were not significantly different between two groups after adjusting for maternal age, BMI, whether embryo transfered with at least one good quality embryo, whether estrogen administration lasted more than 21 days, endometrium thickness at progesterone starting day, progesterone route and newborn gender. The multivariate analysis of LGA and SGA rate for preterm delivery singletons was not performed because the number in this category was too small. After correction for a number of potential confounders, estradiol route was not correlated with infant birthweight (β = -30.962, SE = 68.723, p = 0.653) (Table 5).

Discussion

From our study, oral and vaginal estrogen regimen did not improve clinical pregnancy rate or live birth rate compared to oral estrogen regimen in HRT endometrial preparation FET cycles. Oral and vaginal estrogen regimen induced higher serum E2 level with longer days of estrogen treatment compared to oral estrogen regimen. Adding vaginal estrogen supplement did not change the infant birthweight, SGA, LGA or the preterm delivery rate of the live birth singletons either.

Nowadays, some reports advised better IVF outcomes after freeze-all policy and elective embryo cryopreservation[22-24]. Studies have suggested COS has made a detrimental effect on endometrium receptivity[25, 26]. The supraphysiologic estrogen level impairing embryo-endometrium asynchrony and endometrial receptivity after ovarian stimulation is one of the reasons for the lower implantation rates in fresh transfer cycles compared to FET cycles [27]. Perinatal outcomes such as low birth weight newborn in term singletons have been associated with the hyper-estrogenic milieu generated during COS in fresh embryo transfer[7-11]. Excessive E2 priming induced from development and recruitment of multiple follicles can affect gene expression pattern change like Grb10 and GATA3, and generate epigenetic alterations in developing embryo and fetus through alterations in DNA methylation, histone alteration and others[28-31]. It is also reported that immune environment changed due to the increase of natural killer cells in oocytes during stimulated cycles compared with natural cycles[32]. Ovarian stimulation would probably not benefit implantation, placentation and subsequent fetal growth. Freeze-all policy and FET have showed some advantage of endometrial synchrony, but we must recognize the adverse perinatal outcomes of FET, like higher risk of macrosomia, perinatal mortality and pregnancy complications like pre-eclampsia[33-36]. There is evidence that FET singletons have higher mean birthweight than singletons born after fresh embryo transfer and natural pregnancy[1]. A trend toward higher neonatal death was found in FET group compared to fresh ET group in a RCT of 1508 PCOS patients [37]. FET is not perfect without risks.

Dramatically large-spanned levels of serum estrogen were found between ovarian stimulated cycles and artificial FET cycles. If the supraphysilogic E2 priming was one important reason for the low birth weight and shorter gestational weeks in singletons from stimulated cycles, we wanted to explore the impact of different serum E2 levels induced by different estrogen routes on artificial FET clinical and

neonatal outcomes. Estradiol Femonston has the structure of 17 β-estradiol, which possessed low bio-availability. Only very small part of ingested dose reaches the circulation intact. While the vaginally delivered 17 β-estradiol exerts the local effect on endometrium after vaginal epithelium absorption, bypassing the liver metabolism and achieving higher bioavailability[17]. Estrogen in different routes was prescribed depending on clinical variables like endometrium thickness, previous clinical records and physicians' preference. Considering the rise in FET cycles applied in recent years, it is essential to investigate whether large-spanned levels of peripheral serum estrogen induced by different estradiol routes can impact endometrial receptivity and placentation. There is scarce publication available regarding the effect of vaginally administered estradiol in FET cycles. To our knowledge, one retrospective study of 247 artificial FET cycles has suggested that vaginal estrogen regimen did not promote implantation and pregnancy rate compared to oral regimen. But they didn't investigate the live birth rate[38]. The other prospective study of 78 artificial FET cycles showed vaginal estradiol administration improved endometrial proliferation by eliciting thicker endometrium, but it did not dig and analyze clinical outcomes[39]. Our present study has expanded this concept by providing the comprehensive well-controlled analysis, and evaluated estrogen administration route impact on both live birth rate and neonatal outcomes.

Sekhon et al. suggested duration of estradiol supplementation before progesterone in artificial cycles did not impact frozen blastocyst transfer outcome[13]. This effect may extend beyond the route estradiol absorbed as we demonstrated that estrogen vaginally or orally administered did not impact clinical pregnancy rate and live birth rate in artificial FET cycles. Estrogen administered vaginally and orally together induced higher serum estrogen with longer duration of estrogen supplement, but ended up with thinner endometrium compared to orally administered group. It is noteworthy that the patients in OVE group probably had inferior endometrium proliferation capability, considering their thinner endometrium both in COS. From our result, estrogen vaginally administered didn't improve endometrium thickness and neither increased the successful rate in FET, but was accompanied with the higher circulating estradiol level. The extremely high E2 level might adversely affect the clotting system homeostasis with higher incidence of thromboembolic events[40]. Nevertheless, the high serum E2 priming seemed not to have an impact on singletons birthweight, preterm delivery rate, LGA, and SGA rate.

Our study was not designed to identify the mechanisms underlying the results. The reason why we didn't see the lower birthweight and shorter gestational weeks induced by the high peripheral serum E2 in our cohort may be speculated that the serum E2 level in our analysis is still not high enough to create the supraphysiologic estrogen milieu as in fresh IVF cycles. The mean serum peak E2 value in COS varied from 10460-15362 pmol/L. Estrogen higher than that cutoff value proclaimed the high risk of preterm labor and SGA[7, 11]. The median value of serum E2 level at progesterone starting day in our study was 6106 and 992 pmol/L in two groups respectively, which were both much closer to serum E2 level from natural cycle. Additionally, we didn't use estradiol vaginally replaced of estradiol orally. In OVE group, we still continued the orally administered estradiol and subsequently added estradiol Femoston vaginally in case of vaginal malabsorption in some patients.

The present study has following strengths. The data was collected from one IVF center which guaranteed the same lab equipment and associated operating people and sonographers. This ensured the practice consistency. Male neonates have been predicted to be higher-weighted than female neonates. To control for infant gender and gestational age bias, z score was calculated across different groups. There are two progesterone supplementation routes in this analysis. We accounted for progesterone regimens as potential confounder in multivariate analysis. Progestin has been suggested to directly advance vascular proliferation during placentation[41]. A supraphysiologic progestin exposure in HRT could initiate excessively deep placentation which would make a difference in infant birthweight and obstetrical consequences[42]. Though different progesterone regimens seemed to induce comparable pregnancy rate[43], taking into consideration of progesterone and estrogen regimens simultaneously greatly benefitted in our model. We reviewed the database and restricted the analysis to strict criteria. We tried to minimize any effect associated with uterine factor infertility by excluding patients with endometrium thickness less than 6mm at progesterone starting day and patients who have presented with endometrial polyps, submucosal myomas, endometrium separation or uterus malformation. We didn't include patients with estrogen administered more than 28 days in our study to minimize the effect of extended duration of estrogen, as E2 priming longer than 28 days showed a slacked pregnancy rate in FET[15]. We didn't include blastocyst embryo transfer into the whole cohort to mitigate the possible impact of prolonged in vitro embryo culture on subsequent newborn birthweight and gestational weeks[44, 45]. Only homogenous group of patients who achieved adequate endometrial thickness in their first FET cycles were included to guarantee the relative good-quality embryo transfer and exclude the recurrent FET failure cases, as poor-quality embryos were put forward to be associated with lower birthweight[46]. The long-term follow up of the cohort allowed to provide important neonatal outcomes.

Our study is limited by its retrospective design. Whether and when the estrogen was vaginally administered depended on both patients' endometrium thickness and physicians' preference. Patients added with vaginally administered estradiol are likely to possess inferior capability of endometrium growth and inherent uterine defects that may predispose them to thinner endometrium. The selection bias was possible. We couldn't account for all possible cofounding variables. We couldn't follow up the patient obstetrics details including hypertensive disorders of pregnancy or placental abnormalities, which hampered us to assess the pregnancy risk factors associated to preterm delivery. An evaluation of obstetrics complications such as pre-eclampsia, placenta accrete, previa, pregnancy-induced hypertensive would definitely strengthen our analysis and provide better understanding of high serum estrogen level's impact on placental angiogenesis in late trimester.

Conclusion

This single-center retrospective analysis provided some evidence of the estradiol regimen for women undergoing HRT endometrium preparation FET cycles. Relatively higher serum E2 level induced by orally and vaginally administered estradiol did not increase live birth rate or patients' risk of preterm delivery and low birth weight infants compared to orally administered estradiol. Estrogen vaginally delivered didn't improve endometrium thickness much and neither successful rate in FET, but induced higher serum E2

value with potential maternal undesirable risks. Based on our findings, upon safe and effectiveness consideration, estrogen route could be adjusted to patient preference without compromising FET clinical and neonatal outcomes. Patients should be adequately informed the potential risks and patient's opinion should be taken into consideration in medical practice. Large randomized controlled prospective clinic trials with detailed obstetric follow-up are needed to verify our result and allow a better understanding of the estrogen impact on placental-vessel associated obstetric complications.

Abbreviations

OHSS: ovarian hyperstimulation syndrome

ART: assisted reproductive technology

HRT: hormone replacement therapy

FET: frozen-thawed embryo transfer

COS: controlled ovarian stimulation

IVF: in vitro fertilization

ICSI: intracytoplasmic sperm injection

LGA: large for gestational age

SGA: small for gestational age

95%CI: 95% confidence intervals

OR: odds ratios

BMI: body mass index

Declarations

Ethics approval and consent to participate: Institutional review board and ethics committee of Shanghai General Hospital approval and consent to participate was obtained (2020KY016).

Consent to publication: Informed consent to publication was obtained from all individual participants included in this study.

Availability of data and material: Data available from the corresponding writer if reasonably requested.

Competing interests: Not applicable.

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Authors' contributions:

1. Liu: Project development, data analysis and manuscript writing.
2. Yang: Data analysis and manuscript writing.
3. Sun: Manuscript writing.
4. Zhou: Data collection.
5. Hu: Data collection.
6. Wu: Idea conceiving, project development and manuscript editing.

All authors have read and approved the manuscript.

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References

1. Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, Bhattacharya S: **Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer?** *Hum Reprod Update* 2018, **24**(1):35-58.
2. Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN: **Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique?** *Human reproduction (Oxford, England)* 2014, **29**(3):618-627.
3. Sha T, Yin X, Cheng W, Massey IY: **Pregnancy-related complications and perinatal outcomes resulting from transfer of cryopreserved versus fresh embryos in vitro fertilization: a meta-analysis**. *Fertility and sterility* 2018, **109**(2):330-342 e339.
4. Sites CK, Wilson D, Barsky M, Bernson D, Bernstein IM, Boulet S, Zhang Y: **Embryo cryopreservation and preeclampsia risk**. *Fertility and sterility* 2017, **108**(5):784-790.
5. Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD: **Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan**. *Fertility and sterility* 2014, **101**(1):128-133.
6. Zhang B, Wei D, Legro RS, Shi Y, Li J, Zhang L, Hong Y, Sun G, Zhang T, Li W *et al*: **Obstetric complications after frozen versus fresh embryo transfer in women with polycystic ovary syndrome: results from a randomized trial**. *Fertility and sterility* 2018, **109**(2):324-329.
7. Pereira N, Elias RT, Christos PJ, Petrini AC, Hancock K, Lekovich JP, Rosenwaks Z: **Supraphysiologic estradiol is an independent predictor of low birth weight in full-term singletons born after fresh embryo transfer**. *Hum Reprod* 2017, **32**(7):1410-1417.

8. Zhang W, Ma Y, Xiong Y, Xiao X, Chen S, Wang X: **Supraphysiological serum oestradiol negatively affects birthweight in cryopreserved embryo transfers: a retrospective cohort study**. *Reprod Biomed Online* 2019, **39**(2):312-320.
9. Hu XL, Feng C, Lin XH, Zhong ZX, Zhu YM, Lv PP, Lv M, Meng Y, Zhang D, Lu XE *et al*: **High maternal serum estradiol environment in the first trimester is associated with the increased risk of small-for-gestational-age birth**. *J Clin Endocrinol Metab* 2014, **99**(6):2217-2224.
10. Liu S, Kuang Y, Wu Y, Feng Y, Lyu Q, Wang L, Sun Y, Sun X: **High oestradiol concentration after ovarian stimulation is associated with lower maternal serum beta-HCG concentration and neonatal birth weight**. *Reprod Biomed Online* 2017, **35**(2):189-196.
11. Imudia AN, Awonuga AO, Doyle JO, Kaimal AJ, Wright DL, Toth TL, Styer AK: **Peak serum estradiol level during controlled ovarian hyperstimulation is associated with increased risk of small for gestational age and preeclampsia in singleton pregnancies after in vitro fertilization**. *Fertil Steril* 2012, **97**(6):1374-1379.
12. Jarvela IY, Pelkonen S, Uimari O, Makikallio K, Puukka K, Ruokonen A, Tekay A, Martikainen H: **Controlled ovarian hyperstimulation leads to high progesterone and estradiol levels during early pregnancy**. *Hum Reprod* 2014, **29**(11):2393-2401.
13. Sekhon L, Feuerstein J, Pan S, Overbey J, Lee JA, Briton-Jones C, Flisser E, Stein DE, Mukherjee T, Grunfeld L *et al*: **Endometrial preparation before the transfer of single, vitrified-warmed, euploid blastocysts: does the duration of estradiol treatment influence clinical outcome?** *Fertil Steril* 2019, **111**(6):1177-1185.e1173.
14. Madero S, Rodriguez A, Vassena R, Vernaeve V: **Endometrial preparation: effect of estrogen dose and administration route on reproductive outcomes in oocyte donation cycles with fresh embryo transfer**. *Hum Reprod* 2016, **31**(8):1755-1764.
15. Bourdon M, Santulli P, Kefelian F, Vienet-Legue L, Maignien C, Pocate-Cheriet K, de Mouzon J, Marcellin L, Chapron C: **Prolonged estrogen (E2) treatment prior to frozen-blastocyst transfer decreases the live birth rate**. *Human reproduction (Oxford, England)* 2018, **33**(5):905-913.
16. Rosenwaks Z, Navot D, Veeck L, Liu HC, Steingold K, Kreiner D, Droesch K, Stumpf P, Muasher SJ: **Oocyte donation. The Norfolk Program**. *Ann N Y Acad Sci* 1988, **541**:728-741.
17. Paulson RJ: **Hormonal induction of endometrial receptivity**. *Fertil Steril* 2011, **96**(3):530-535.
18. Zhang J, Liu H, Mao X, Chen Q, Si J, Fan Y, Xiao Y, Wang Y, Kuang Y: **Effect of endometrial thickness on birthweight in frozen embryo transfer cycles: an analysis including 6181 singleton newborns**. *Human reproduction (Oxford, England)* 2019, **34**(9):1707-1715.
19. **The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting**. *Human reproduction (Oxford, England)* 2011, **26**(6):1270-1283.
20. Rodriguez-Purata J, Lee J, Whitehouse M, Duke M, Grunfeld L, Sandler B, Copperman A, Mukherjee T: **Reproductive outcome is optimized by genomic embryo screening, vitrification, and subsequent transfer into a prepared synchronous endometrium**. *J Assist Reprod Genet* 2016, **33**(3):401-412.

21. Dai L, Deng C, Li Y, Zhu J, Mu Y, Deng Y, Mao M, Wang Y, Li Q, Ma S *et al*: **Birth weight reference percentiles for Chinese**. *PloS one* 2014, **9**(8):e104779.
22. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C: **Clinical rationale for cryopreservation of entire embryo cohorts in lieu of fresh transfer**. *Fertil Steril* 2014, **102**(1):3-9.
23. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S: **Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfers in high responders**. *Fertil Steril* 2011, **96**(2):516-518.
24. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S: **Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders**. *Fertil Steril* 2011, **96**(2):344-348.
25. Mirkin S, Nikas G, Hsiu JG, Diaz J, Oehninger S: **Gene expression profiles and structural/functional features of the peri-implantation endometrium in natural and gonadotropin-stimulated cycles**. *J Clin Endocrinol Metab* 2004, **89**(11):5742-5752.
26. Basir GS, O WS, Ng EH, Ho PC: **Morphometric analysis of peri-implantation endometrium in patients having excessively high oestradiol concentrations after ovarian stimulation**. *Hum Reprod* 2001, **16**(3):435-440.
27. Weinerman R, Mainigi M: **Why we should transfer frozen instead of fresh embryos: the translational rationale**. *Fertil Steril* 2014, **102**(1):10-18.
28. Horcajadas JA, Riesewijk A, Polman J, van Os R, Pellicer A, Mosselman S, Simon C: **Effect of controlled ovarian hyperstimulation in IVF on endometrial gene expression profiles**. *Mol Hum Reprod* 2005, **11**(3):195-205.
29. Horcajadas JA, Minguez P, Dopazo J, Esteban FJ, Dominguez F, Giudice LC, 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**(11):4500-4510.
30. Haouzi D, Assou S, Mahmoud K, Tondeur S, Reme T, Hedon B, De Vos J, Hamamah S: **Gene expression profile of human endometrial receptivity: comparison between natural and stimulated cycles for the same patients**. *Hum Reprod* 2009, **24**(6):1436-1445.
31. Lee B, Kroener LL, Xu N, Wang ET, Banks A, Williams J, 3rd, Goodarzi MO, Chen YI, Tang J, Wang Y *et al*: **Function and Hormonal Regulation of GATA3 in Human First Trimester Placentation**. *Biol Reprod* 2016, **95**(5):113.
32. Junovich G, Mayer Y, Azpiroz A, Daher S, Iglesias A, Zylverstein C, Gentile T, Pasqualini S, Markert UR, Gutierrez G: **Ovarian stimulation affects the levels of regulatory endometrial NK cells and angiogenic cytokine VEGF**. *Am J Reprod Immunol* 2011, **65**(2):146-153.
33. Wennerholm UB, Henningsen AK, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, Forman J, Gissler M, Nygren KG, Tiitinen A: **Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group**. *Hum Reprod* 2013, **28**(9):2545-2553.

34. Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, Zhu Y, Deng X, Qi X, Li H *et al*: **Transfer of Fresh versus Frozen Embryos in Ovulatory Women**. *N Engl J Med* 2018, **378**(2):126-136.
35. Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C: **Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos**. *Hum Reprod* 2012, **27**(5):1343-1350.
36. Opdahl S, Henningsen AA, Tiitinen A, Bergh C, Pinborg A, Romundstad PR, Wennerholm UB, Gissler M, Skjaerven R, Romundstad LB: **Risk of hypertensive disorders in pregnancies following assisted reproductive technology: a cohort study from the CoNARTaS group**. *Hum Reprod* 2015, **30**(7):1724-1731.
37. Chen ZJ, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, Yang J, Liu J, Wei D, Weng N *et al*: **Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome**. *N Engl J Med* 2016, **375**(6):523-533.
38. Liao X, Li Z, Dong X, Zhang H: **Comparison between oral and vaginal estrogen usage in inadequate endometrial patients for frozen-thawed blastocysts transfer**. *International journal of clinical and experimental pathology* 2014, **7**(10):6992-6997.
39. Fanchin R, Righini C, Schönauer LM, Olivennes F, Cunha Filho JS, Frydman R: **Vaginal versus oral E(2) administration: effects on endometrial thickness, uterine perfusion, and contractility**. *Fertility and sterility* 2001, **76**(5):994-998.
40. Magnusson A, Kallen K, Thurin-Kjellberg A, Bergh C: **The number of oocytes retrieved during IVF: a balance between efficacy and safety**. *Human reproduction (Oxford, England)* 2018, **33**(1):58-64.
41. Walter LM, Rogers PA, Girling JE: **The role of progesterone in endometrial angiogenesis in pregnant and ovariectomised mice**. *Reproduction (Cambridge, England)* 2005, **129**(6):765-777.
42. Pirtea P, de Ziegler D, Ayoubi JM: **Implantation rates of euploid embryos are not influenced by the duration of estradiol priming, but the hormonal environment-estradiol and progesterone-may affect placentation**. *Fertil Steril* 2019, **111**(6):1117-1118.
43. Shapiro DB, Pappadakis JA, Ellsworth NM, Hait HI, Nagy ZP: **Progesterone replacement with vaginal gel versus i.m. injection: cycle and pregnancy outcomes in IVF patients receiving vitrified blastocysts**. *Human reproduction (Oxford, England)* 2014, **29**(8):1706-1711.
44. Zhang J, Wang Y, Liu H, Mao X, Chen Q, Fan Y, Xiao Y, Kuang Y: **Effect of in vitro culture period on birth weight after vitrified-warmed transfer cycles: analysis of 4,201 singleton newborns**. *Fertil Steril* 2019, **111**(1):97-104.
45. Ginstrom Ernstad E, Spangmose AL, Opdahl S, Henningsen AA, Romundstad LB, Tiitinen A, Gissler M, Wennerholm UB, Pinborg A, Bergh C *et al*: **Perinatal and maternal outcome after vitrification of blastocysts: a Nordic study in singletons from the CoNARTaS group**. *Hum Reprod* 2019, **34**(11):2282-2289.
46. Zhang J, Huang J, Liu H, Wang B, Yang X, Shen X, Mao X, Wang Y, Kuang Y: **The impact of embryo quality on singleton birthweight in vitrified-thawed single blastocyst transfer cycles**. *Human reproduction (Oxford, England)* 2020, **35**(2):308-316.

Tables

Table 1: Baseline demographic and cycle characteristics according to estrogen regimens

	OVE group (N=324)	OE group (N=468)	P
rnal age (year)	30.87 ± 4.51	30.75 ± 4.5	0.717
	21.157 ± 2.91	21.57 ± 3.2	0.059
ast one good quality embryo	282 (87.04%)	407 (86.97%)	0.977
of estradiol administration			
1 days	35	5	<0.001
l days	289	463	
metrium thickness at progesterone starting mm)	8.75 (8.200, 9.325)	9 (8.6, 9.7)	<0.001
metrium thickness at trigger day in COS (mm)	8.85 (7.4, 10.0) (N=306)	9.8 (7.8, 12.0) (N=441)	<0.001
esterone route			
ramuscular	115	193	0.103
ginal	209	275	
vel at progesterone starting day (pmol/L)	6105.5 (2234.00, 9065.25) (N=292)	992.5 (644.50, 1400.75) (N=444)	<0.001
vel at day 14 after transfer (pmol/L)	1346 (969,1884) (N=257)	1420 (1038,1875) (N=373)	0.364
el at progesterone starting day (pmol/L)	1.11 (0.67, 1.66) (N=291)	1.17(0.68,1.79) (N=442)	0.375

: Data are presented as mean±SD for continuous variables in formal distribution, median quartile, third quartile) for continuous variables in informal distribution. P values were sed with the use of t tests or Mann-Whitney U test or chi-square. OVE group stands for diol orally and vaginally administered group. OE group stands for estradiol orally nistered group. COS stands for controlled ovarian stimulation.

Table 2 Clinical outcomes according to different estrogen routes

	OVE group (N=324)	OE group (n=468)	Crude OR (95%CI)	Adjusted OR (95%CI)
cal pregnancy	157（48.46%）	200（42.74%）	1.260 (0.948-1.675)	1.278 (0.937-1.743)
birth	127（39.2%）	161（34.4%）	1.229 (0.917-1.649)	1.327 (0.982-1.794)

: Analyses were adjusted for maternal age, BMI, whether days of estrogen treatment > 21, her at least one good quality embryo was transferred, endometrium thickness at metrium transform day, progesterone regimen. OE group stands for estradiol orally nistered group. COS stands for controlled ovarian stimulation. CI=confidence interval; OR=odds

Table 3　Baseline demographics, cycle characteristics and neonatal outcomes of live birth singletons according to different estradiol routes

	Vaginal and oral (N=98)	Oral only (N=130)	P
rnal age (year)	29.96 ± 3.83	30.58 ± 3.95	0.236
	21.3 ± 2.91	21.48 ± 3.12	0.660
ast one good quality embryo	90	122	0.923
metrium thickness at progesterone starting day)	8.8 (8.2, 9.4)	9.0 (8.6, 9.9)	0.004
metrium thickness at trigger day in COS (mm)	9 (8,10)	9.75 (6.75,11.70)	0.100
of estradiol administration			
1 days	12	1	0.001
1 days	86	129	
vel at progesterone starting day (pmol/L)	5977 (1882, 9043)	962(647.75,1364.50)	<0.001
	(N=89)	(N=120)	
esterone route			
ramuscular	34	57	0.162
ginal	64	73	
ational Age			
37	5	8	0.735
7	93	122	
weight	3222 ± 215.43	3209.67 ± 228.09	0.679
ore	0.35 ± 0.89	0.38 ± 1.12	0.793
ational age > 37weeks	93	122	0.735
New born gender			
Female	44	60	0.786
Male	49	62	
Birthweight (g)	3411.72 ± 379.45	3415.06 ± 461.54	0.954
SGA	2	5	0.693
LGA	18	21	0.660

e: Data are presented as mean±SD for continuous variables in formal distribution, ian (first quartile, third quartile) for continuous variables in informal distribution. P es were assessed with the use of t tests or Mann-Whitney U tests or chi-square her's exact tests as appropriate). COS stands for controlled ovarian stimulation. SGA ds for small for gestational age. LGA stands for large for gestational age.

Table 4: Crude and adjusted ORs of birthweight categories in singleton births.

	Crude OR (95%CI)	Adjusted OR (95%CI)
Preterm delivery	1.220 (0.386-3.850)	0.969 (0.292-3.214)
ational age >37 weeks		
LGA	1.168 (0.584-2.334)	1.165 (0.545-2.490)
SGA	0.521 (0.099-2.743)	0.569 (0.096-3.369)

: Analyses were adjusted for maternal age, BMI, transfer with at least one good quality embryo, endometrium ness, whether estrogen administration >21 days, progesterone route, newborn gender. CI stands for confidence val; OR stands for odds ratio. SGA stands for small for gestational age. LGA stands for large for tational age.

Table 5: Multiple linear regression analysis of birth weight among live birth singletons.

Model

	Unstandardised coefficients		Standardised coefficients	t	P value
	B	Std.error	Beta		
Constant	4154.212	464.787		8.938	<0.001
Maternal age	-16.475	8.388	-0.133	-1.964	0.051
BMI	-10.706	10.896	-0.067	-0.983	0.327
Estradiol vaginally and orally (vs orally only)	-30.962	68.723	-0.032	-0.451	0.653
Whether days of estradiol administration > 21 days	109.875	147.750	0.053	0.744	0.458
Endometrium thickness	-5.741	32.120	-0.0313	-0.179	0.858
At least one good quality embryo	-34.886	128.221	-0.018	-0.272	0.786
Progesterone intramuscular (vs vaginal	-34.175	66.569	-0.035	-0.513	0.608
Newborn Male (vs female)	67.472	65.651	0.070	1.028	0.305

Figures

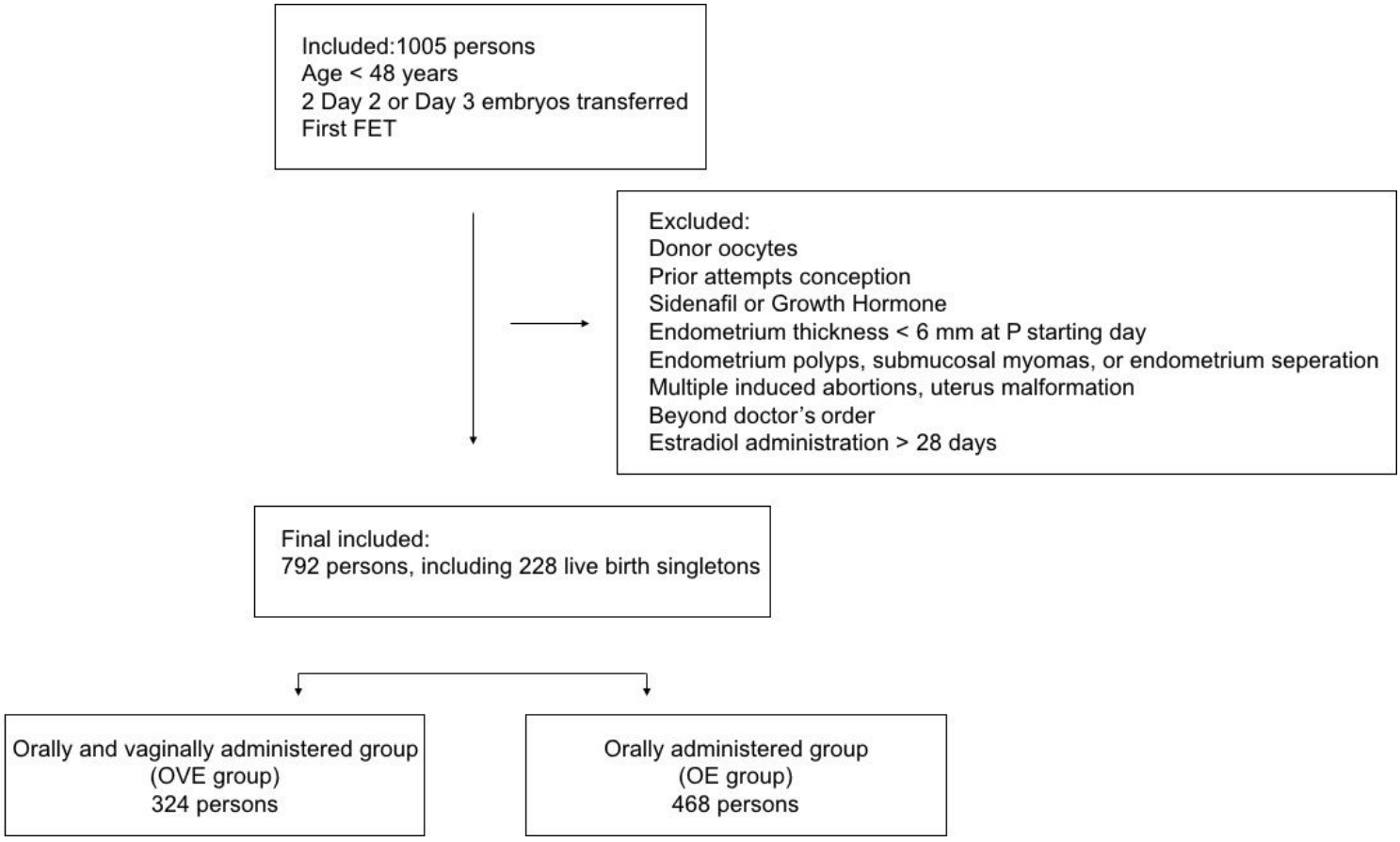

Figure 1

Flow diagram of patient inclusion.

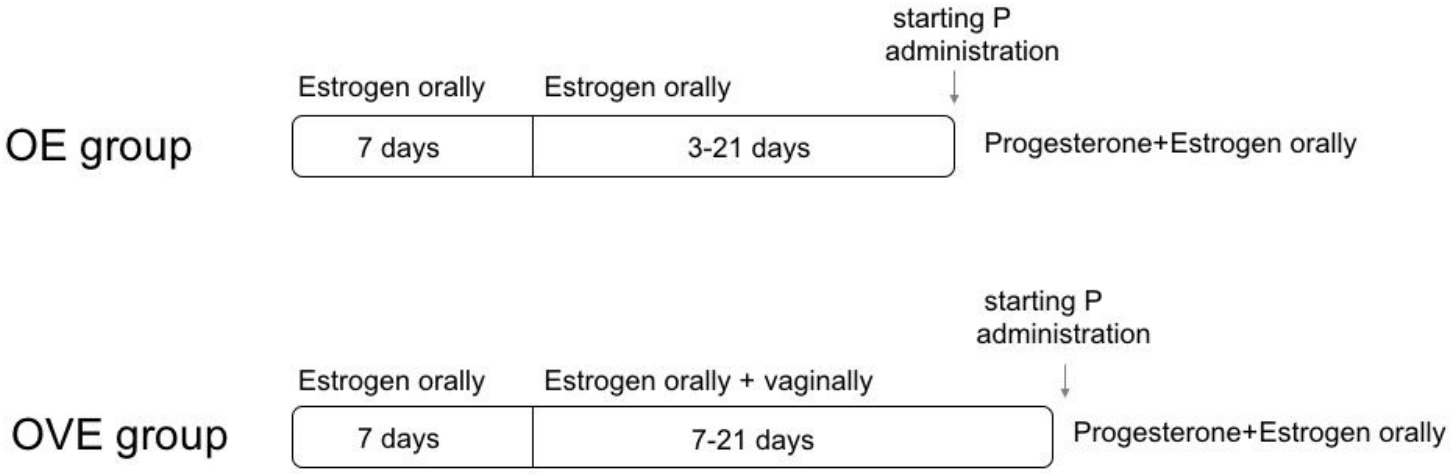

Figure 2

Estrogen priming flow.

Supplementary Files

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