Pape Janna (Orcid ID: 0000-0002-7858-2288)

Hormone replacement cycles are associated with a higher risk of hypertensive disorders: retrospective cohort study in singleton and twin pregnancies

Running title: Hypertensive disorders in HRC-FET

Janna Pape[1]*, Jérémy Levy[2], Michael von Wolff[1]

[1] University Women's Hospital, Division of Endocrinology and Reproductive Medicine, Inselspital, 3010 Bern, Switzerland

[2] FIVNAT statistician, Swiss Society for Reproductive Medicine, 5001 Aarau, Switzerland

*Corresponding author: Address: Theodor-Kocher-Haus, Friedbühlstrasse 19, 3010 Bern

E-Mail: janna.pape@insel.ch, Tel.: +41 77 960 16 28

ABSTRACT

Objective: To elaborate the associations of different cycle regimens (natural cycle = NC, stimulated cycle = SC, hormone replacement cycle = HRC) on maternal and neonatal adverse pregnancy outcomes after frozen-thawed embryo transfers (FET).

Design: Population-based registry study.

Setting: Swiss IVF Registry.

Population or Sample: Singleton (n = 4636) and twin life births (n = 544) after NC-FET (n = 776), SC-FET (n = 758) or HRC-FET (n = 3646) registered from 2014 to 2019.

Methods: Fifteen pregnancy pathologies were modelized for singleton and twin pregnancies using mixed models adjusted for cycle regimen, delivery, fertilization technique, chronic anovulation, age of mother and centre.

Main Outcome Measures: Maternal (vaginal bleeding, isolated arterial hypertension and preeclampsia) and neonatal (gestational age, birthweight, mode of delivery) adverse pregnancy outcomes.

Results: In singleton pregnancies, the incidences of bleeding in first trimester, isolated hypertension and preeclampsia were highest in HRC-FET with doubled odds of bleeding in first trimester (adjusted odds ratio = aOR 2.23; 95% CI 1.33-3.75), isolated hypertension (aOR 2.50; 95% CI 1.02-6.12) and preeclampsia (aOR 2.16; 95% CI 1.13-4.12) in HRC-FET vs. NC-FET and with doubled respectively sixfold odds of bleeding (aOR 2.08; 95% CI 1.03-4.21) and preeclampsia (6.02; 95% CI 1.38-26.24) in HRC-FET vs. SC-FET. In twin pregnancies, the incidence of preeclampsia was highest in HRC-FET with numerically higher odds of preeclampsia in HRC-FET vs. NC-FET and vs. SC-FET.

Conclusions: Our data implied the highest maternal risks of hypertensive disorders in HRC-FET, therefore clinicians should prefer SC-FET or NC-FET if medically possible.

Funding: Public universities.

Keywords: Frozen-thawed embryo transfer, cycle regimen, hypertensive disorder, preeclampsia, twin pregnancy

FUNDING

Statistical calculation was financially supported by IBSA Institut Biochimique SA, which did not have any role in designing or conducting the study and the calculations, nor in the decision on preparation or publication of the manuscript.

INTRODUCTION

Frozen-thawed embryo transfers (FET) are a key component of assisted reproductive technologies (ART) [1, 2] and has increased markedly to 27% of all cycles in Europe [3, 4, 5]. Various cycle regimens are used worldwide due to insufficient evidence to favour particular transfer schedules [6]. In general, FET can be performed in Hormone Replacement Cycles (HRC-FET), low-dose Stimulation Cycles (SC-FET) or Natural Cycles (NC-FET) [7]. HRC-FET is medically necessary in amenorrhea or irregular cycles. SC-FET can also be applied in irregular cycles; however, it is less frequently used since daily and expensive gonadotropin injections are required. Practically, HRC-FET offers greater flexibility in scheduling blastocyst thawing, which may be beneficial for both the patient and the IVF-clinic.

No differences between these cycle regimens have been demonstrated in terms of pregnancy rates [6]. However, serious maternal and neonatal complications associated with HRC-FET were first described in data from Sweden [8], Japan [9] and China [10]: A doubled to tripled risk of preeclampsia [8-10], a sixfold risk of placenta accreta [9, 11] and doubled risk of caesarean section [11] occurred in HRC-FET compared to NC-FET. A recent systematic review and metaanalysis revealed lowest risks of hypertensive disorders in pregnancy (RR 0.61, 95% CI 0.50-0.73) and preeclampsia (RR 0.47, 95% CI 0.42-0.53) in NC-FET compared to HRC-FET [12]. Data of SC-FET were comparable to NC-FET showing no increased adverse maternal or neonatal outcomes [8, 13].

The inhibition of follicles and luteal bodies [14, 15], altered progesterone [16, 17] and supraphysiological estrogen levels [18] in HRC-FET cycles may lead to the abovementioned

pregnancy complications. An insufficient cardiovascular adaption was observed in women without corpus luteum [19], which may be caused by the lack of circulating vasoactive hormones released by the corpus luteum [20].

So far, the great proportion of register studies has been conducted in singleton pregnancies and data is also poor for SC-FET. Regarding the increasing rate of HRC-FET cycles worldwide, it is essential to elaborate the associations of each cycle regimen on maternal and neonatal adverse pregnancy outcomes, not only in singletons but also in in twin pregnancies.

METHODS

Study population

We conducted a retrospective cohort study collecting singleton and twin births after FET that were registered in the Swiss ART Registry from 2014 to 2019. Inclusion criterion was live birth after FET. Exclusion criteria were stillbirths.

Women were divided into three groups according to the different cycle regimens for endometrial preparation, which were defined as follows:

- NC-FET: Natural cycle with or without hCG ovulation trigger.
- SC-FET: Women treated with low-dose ovarian stimulation (recombinant and human menopause gonadotropin with or without gonadotropin-releasing hormone agonist / antagonist) and with or without luteal phase support.
- HRC-FET: Women who received estradiol and progesterone to stimulate endometrial growth and transformation.

Outcomes

Maternal outcomes included pregnancy complications, e.g. bleeding in first, second and third trimester, premature labour, premature rupture of membranes, placenta previa, isolated hypertension (>140/90 mmHg), preeclampsia, intrauterine growth restriction and gestational diabetes.

Neonatal outcomes comprised gestational age with pre- and post-term births, weight at birth with the proportion of small and large for gestational age and mode of delivery.

Statistical Analysis

Data were analysed by cycle regimens (NC-FET, SC-FET, HRC-FET) for the entire population or in singleton and twin pregnancies. Descriptive statistics were used to present patients and cycles characteristics, maternal and neonatal outcomes. Adjusted odds ratios with pregnancy complications as outcome and cycle regimen, fertilization technique, age of mother, polycystic ovary syndrome (PCOS) and chronic anovulation as fixed effects and subcentre ID (n = 71) as random effect were also calculated.

None of the *P*-values generated for the analysis were corrected for multiple testing; p-values are therefore nominal and need to be interpreted accordingly. All analyses were performed with SAS 9.4.

RESULTS

Our study cohort comprised a total of 4636 singleton and 544 twin births (corresponding to 1088 twins) which were distributed into the three groups: NC-FET (n = 776), SC-FET (n = 758) and HRC-FET (n = 3646).

The mean maternal age was 35.3, 35.3 and 35.1 years in the NC-FET, SC-FET and HRC-FET group respectively. The proportion of previous recurrent miscarriages was overall low (NC: 0.3%, SC: 0.3%, HRC: 0.7%). The FET groups differed in the proportion of chronic anovulation or PCOS (NC: 5.9%, SC: 10.0%, HRC: 17.3%), and severe endometriosis (NC: 3.6%, SC: 3.8%, HRC: 5.8%). Except for thyroid disease (NC: 3.6%, SC: 3.4%, HRC: 5.8%), there were no differences in comorbidities. The largest proportion of single embryo transfers were conducted in HRC-FET (56.0%) and double embryo transfers in SC-FET (55.0%). Numbers of triple embryo transfers were overall low with the highest rate in SC-FET (7.0%). Day of embryo transfer (day 2 / 3 or day 5) was not documented in the registry. Maternal characteristics separated in singleton and twin deliveries were comparable (Table I).

Singleton pregnancies

Differences between the cycle regimens with highest incidences in HRC-FET were observed in bleeding in the first trimester (NC: 2.8%, SC: 2.6%, HRC: 7.0%), premature rupture of membranes (NC: 1.4%, SC: 1.4%, HRC: 3.1%), isolated hypertension (NC: 0.9%, SC: 0.2%, HRC: 1.8%) and preeclampsia (NC: 1.7%, SC: 0.3%, HRC: 2.8%) (Table II). In SC-FET, gestational diabetes occurred most (NC: 4.6%, SC: 6.9%, HRC: 4.5%) and intrauterine growth restriction (IUGR) least frequently (NC: 1.8%, SC: 0.2%, HRC: 1.3%). There were no differences in the incidences of bleeding in the 2. and 3. trimester, premature labour in the second trimester, placenta previa, cervical insufficiency with cerclage, hospitalisation in pregnancy and cholestasis between cycle regimens. The registry choice “other pregnancy complications” was different between the groups and lowest in HRC-FET (NC: 47.9%, SC: 42.0%, HRC: 28.5%) (Table II).

Multivariate analysis revealed doubled odds of bleeding in the first trimester (aOR 2.23; 95% CI 1.33-3.75), isolated hypertension (aOR 2.50; 95% CI 1.02-6.12) and preeclampsia (aOR 2.16; 95% CI 1.13-4.12) in HRC-FET compared to NC-FET. There were doubled odds of bleeding in first trimester (aOR 2.08; 95% CI 1.03-4.21) and even sixfold odds of preeclampsia in HRC-FET compared to SC-FET (aOR 6.02; 95% CI 1.38-26.24). The odds of developing gestational diabetes were lower in HRC-FET (aOR 0.51; 95% CI 0.30-0.88) compared to SC-FET. NC-FET and SC-FET revealed comparable odds in most cases (Table II).

Overall, neonatal outcomes including gestational age, the proportion of pre- and post-term births and birthweight were similar in the three FET groups. Differences were shown in the mode of delivery: Highest caesarean sections rates were reported in HRC-FET (NC: 38.4%, SC: 44.3%, HRC: 51%) and highest spontaneous birth rates in NC-FET (NC: 51.2%, SC: 45.0%, HRC: 33.8%) (Table IV).

Twin pregnancies

Difference between the cycle regimens with highest incidence in HRC-FET was observed in preeclampsia (NC: 2.7%, SC: 1.0%, HRC: 7.2%). Similar to singleton pregnancies, IUGR occurred least frequently in SC-FET (NC: 8.2%, SC: 0%, HRC: 2.9%). There were no relevant differences in any other incidences of pregnancy outcomes by cycle regimens (Table III).

Multivariate analysis showed numerically higher odds for pregnancy complications in HRC-FET. The odds of preeclampsia doubled compared to NC-FET (aOR 2.54; 95% CI 0.54-11.94) and multiplied compared to SC-FET (aOR 4.05; 95% CI 0.47-34.74), while the odds of bleeding in the first trimester increased even fivefold in HRC-FET compared to SC-FET (aOR 5.52; 95% CI 0.54-56.43) (Table III).

Similar to singletons, there were no differences in neonatal outcomes, but twins were mainly born by caesarean section in all cycle regimens (NC: 86.3%, SC: 86.5%, HRC: 80.3%).

DISCUSSION

This study shows an association between hypertensive disorders and HRC-FET. We found highest incidences of bleeding in the first trimester, isolated hypertension and preeclampsia in singleton pregnancies. To our knowledge, this is the first study to investigate the associations of pregnancy outcomes among different cycle regimens also in a large cohort of SC-FET and twin pregnancies.

Previous studies have found that HRC-FET is one important risk factor for hypertensive disorders [8, 9, 21-23]. Conflicting results are described in neonatal outcomes including lack of statistical differences between cycle regime [24] but also higher proportions of post-term deliveries [11], macrosomia [8] and large for gestational age babies [25-27] in HRC-FET. These divergent outcomes may be explained by different sample sizes or various baseline characteristics of the cohorts, especially in the percentage of women with PCOS.

Our cohort revealed a high proportion of HRC-FET both in singleton (70.6%) and twin pregnancies (68.9%) which is comparable to Japan (72%) [9] and far higher compared to Sweden (15%) [8] and Denmark (31%) [23]. These register studies mainly analysed singleton deliveries (Asserhøj et al., 2021, Ginström Ernstad et al., 2019, Makhijani, et al., 2020, Wang

et al., 2020, Zong, et al., 2020) or restricted the sensitivity analysis to singletons (Saito et al., 2019). Furthermore, the majority of studies compared HRC-FET with NC-FET [9, 22, 28] and cycle regimens were defined differently: The Swedish study [8] defined SC-FET as natural cycles with ovulation trigger. The Danish study [23] separated the groups into natural cycles with (= modified NC-FET) or without ovulation trigger (= true NC-FET). In our study, SC-FET comprised all methods of low-dose ovarian stimulation and NC-FET was defined by lack of ovarian stimulation.

In our analysis, we not only confirmed the higher risk profile in FET regimes without corpus luteum showing increased risks of hypertensive disorders but additionally added the following findings:

In singleton pregnancies, bleeding in the first trimester occurred more often in NC-FET and multivariate analysis revealed doubled odds in HRC-FET compared to NC-FET: Excess estradiol levels in the early stage of pregnancy have been shown to have adverse effects on placentation, causing cell death, inhibiting trophoblast invasion in cytotrophoblast and placental cell lines [29] which might be reflected by frequent bleedings. Interestingly, gestational diabetes occurred more often in SC-FET compared to HRC-FET. Herby, decreased secretion of insulin-counteracting hormones from the placenta is discussed to suppress the pathogenesis of gestational diabetes in some HRC-FET-derived pregnancies [30, 31].

In twin pregnancies, the incidence of preeclampsia was also highest in HRC-FET in the multivariate analysis (Table III); however, the overall low absolute numbers of complications might explain this statistical result.

Regarding neonatal outcomes, we found highest caesarean section rate in HRC-FET and highest spontaneous birth rate in NC-FET. The higher percentage of pregnancy complications might be one important reason for the higher proportion of caesarean sections in HRC-FET. Twins are usually delivered by caesarean section because of inherent higher obstetrics risks, which could explain the lack of differences between the cycle regimes (Table IV).

Strengths and limitations

The great strength of our study is the large cohort of singleton (n = 4636) and twin pregnancies (n = 544) after three different cycle regimes, representing the total Swiss ART data of the years 2014 – 2019. The use of the Swiss ART data registry is both one strength as well as the main limitation of our analysis: Studies based on registry data are often accompanied by selection bias (nonrandomized) and missing data (lack of documentation). The data are observational in nature and it is possible that treatment patterns (unmeasured confounders) might be responsible for the observed associations. Furthermore, with the large number of outcomes, some observed associations might have occurred by chance and might not reflect an existing relationship. In the current analysis selection bias occurred in unequally distributed maternal characteristics such as PCOS and in treatment type (Table I). Additionally, undocumented, background characteristics might have had an impact on the clinician's choice of treatment method. Potential confounders like BMI, history of hypertension or preeclampsia [32, 33] were not documented and could not be considered while analysing the data. Due to the positive correlation between PCOS and BMI, it is possible that the HRC-FET group comprised a larger proportion of women with higher BMI. However, the relative proportion of PCOS women in HRC-FET was overall small (17.3%) and cannot explain the far higher application of HRC-FET cycles (70.4%) and therefore the pregnancy complications. So, it can be assumed that most normoovulatory women also received HRC-FET for practical reasons. Moreover, we were able to adjust for PCOS in the multivariate analysis.

The data also shows a large amount of pregnancies with "other pregnancy complications". A supportive analysis was conducted excluding centres with more than 40% of "other" or less than 10% of specified pregnancy complications. As the results on maternal and neonatal outcomes were comparable in this approach, a bias by inaccurate documentation could be excluded.

Some studies also question whether PGT-A may increase the risk of preeclampsia or gestational hypertension [34, 35]. PGT has been legally permitted in Switzerland at the end of 2017 and was slowly introduced during the following years. Therefore, no PGT data are available for the analysis period.

Additionally, a quantification of blood loss and / or bleeding episodes would have been interesting to analyse but were not documented in the registry. This aspect should be investigated in prospective cohort studies.

CONCLUSION

This is the first large register study to demonstrate an association between the three different cycle regimes including a large proportion of SC-FET and twin pregnancies.

Our data showed higher odds of bleeding, isolated hypertension and preeclampsia in patients conceiving after HRC-FET compared to NC-FET and SC-FET, indicating that these risks might be associated with the inhibition of the luteal body development. In twin pregnancies, the incidence of preeclampsia was also higher. Prospective randomised controlled trials [36] are essential to clarify the potential mechanism underlying the influence of FET regimes with or without corpus luteum affecting pregnancy complications.

DISCLOSURE OF INTEREST

J.L.: Payment for statistical analysis and revision of the manuscript (payment by v.W.). J.P and v.W. declare that they have no conflict of interest.

CONTRIBUTION TO AUTHORSHIP

All authors met conditions for authorship. J.P. interpreted the data, drafted the first version of the article and revised the manuscript. J.L. provided critical inputs on the study design, performed statistical analyses and revised the manuscript. v.W. conceptualized the study, interpreted the data and revised the manuscript. All authors edited and approved the final version for publication.

DETAILS OF ETHIC APPROVAL

Each of the 29 Swiss ART centres was informed about the use of the health-related personal data collected in the registry and gave consent for this research project. The local ethics board approved the protocol (Project-ID: 2021-01671).

DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

ACKNOWLEDGEMENT

We thank all 29 Swiss ART centres who gave consent for this research project. We thank the Institut Biochimique SA, Lugano for financial support and Dr. Elizabeth Krämer for linguistic revision.

REFERENCES

1. Li Z, Wang AY, Bowman M, Hammarberg K, Farquhar C, Johnson L, et al. Cumulative live birth rates following a 'freeze-all' strategy: a population-based study. Human reproduction open. 2019;2019(2):hoz004.
2. Zaat T, Zagers M, Mol F, Goddijn M, van Wely M, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database Syst Rev. 2021 Feb 4;2(2):Cd011184.
3. Nygren KG, Andersen AN. Assisted reproductive technology in Europe, 1998. Results generated from European registers by ESHRE. European Society of Human Reproduction and Embryology. Human reproduction (Oxford, England). 2001 Nov;16(11):2459-71.
4. Ferraretti AP, Nygren K, Andersen AN, de Mouzon J, Kupka M, Calhaz-Jorge C, et al. Trends over 15 years in ART in Europe: an analysis of 6 million cycles. Human reproduction open. 2017;2017(2):hox012.
5. Wyns C, De Geyter C, Calhaz-Jorge C, Kupka MS, Motrenko T, Smeenk J, et al. ART in Europe, 2017: results generated from European registries by ESHRE. Human reproduction open. 2021;2021(3):hoab026.
6. Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Rev. 2017;7(7):CD003414-CD.
7. Mackens S, Santos-Ribeiro S, van de Vijver A, Racca A, Van Landuyt L, Tournaye H, et al. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. Human reproduction (Oxford, England). 2017 Nov 1;32(11):2234-42.
8. Ginström Ernstad E, Wennerholm UB, Khatibi A, Petzold M, Bergh C. Neonatal and maternal outcome after frozen embryo transfer: Increased risks in programmed cycles. American journal of obstetrics and gynecology. 2019 Aug;221(2):126.e1-.e18.
9. Saito K, Kuwahara A, Ishikawa T, Morisaki N, Miyado M, Miyado K, et al. Endometrial preparation methods for frozen-thawed embryo transfer are associated with altered risks of hypertensive disorders of pregnancy, placenta accreta, and gestational diabetes mellitus. Human Reproduction. 2019;34(8):1567-75.
10. Wang B, Zhu Q, Wang Y. Pregnancy Outcomes After Different Cycle Regimens for Frozen-Thawed Embryo Transfer: A Retrospective Study Using Propensity Score Matching. 2020 2020-July-28;7(327).
11. Saito K, Miyado K, Yamatoya K, Kuwahara A, Inoue E, Miyado M, et al. Increased incidence of post-term delivery and Cesarean section after frozen-thawed embryo transfer during a hormone replacement cycle. J Assist Reprod Genet. 2017 Apr;34(4):465-70.
12. Zhao D, Zhao G, Fan J, Chen H, Lopriore E, Li X. Live birth rate of twin pregnancies after frozen embryo transfer: natural cycle versus ovulation induction regimens. Archives of gynecology and obstetrics. 2021 Sep;304(3):619-26.
13. Zong L, Liu P, Zhou L, Wei D, Ding L, Qin Y. Increased risk of maternal and neonatal complications in hormone replacement therapy cycles in frozen embryo transfer. Reprod Biol Endocrinol. 2020;18(1):36-.
14. Conrad KP, Baker VL. Corpus luteal contribution to maternal pregnancy physiology and outcomes in assisted reproductive technologies. American journal of physiology Regulatory, integrative and comparative physiology. 2013 Jan 15;304(2):R69-72.
15. von Versen-Höynck F, Narasimhan P, Selamet Tierney ES, Martinez N, Conrad KP, Baker VL, et al. Absent or Excessive Corpus Luteum Number Is Associated With Altered Maternal Vascular Health in Early Pregnancy. Hypertension. 2019;73(3):680-90.
16. Tamimi R, Lagiou P, Vatten LJ, Mucci L, Trichopoulos D, Hellerstein S, et al. Pregnancy hormones, pre-eclampsia, and implications for breast cancer risk in the offspring. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2003 Jul;12(7):647-50.
17. Jauniaux E, Jurkovic D. Placenta accreta: pathogenesis of a 20th century iatrogenic uterine disease. Placenta. 2012 Apr;33(4):244-51.

18. Bonagura TW, Pepe GJ, Enders AC, Albrecht ED. Suppression of extravillous trophoblast vascular endothelial growth factor expression and uterine spiral artery invasion by estrogen during early baboon pregnancy. Endocrinology. 2008 Oct;149(10):5078-87.
19. Versen-Höynck Fv, Schaub AM, Chi Y-Y, Chiu K-H, Liu J, Lingis M, et al. Increased Preeclampsia Risk and Reduced Aortic Compliance With In Vitro Fertilization Cycles in the Absence of a Corpus Luteum. 2019;73(3):640-9.
20. von Versen-Höynck F, Strauch NK, Liu J, Chi YY, Keller-Woods M, Conrad KP, et al. Effect of Mode of Conception on Maternal Serum Relaxin, Creatinine, and Sodium Concentrations in an Infertile Population. Reproductive sciences (Thousand Oaks, Calif). 2019 Mar;26(3):412-9.
21. Jing S, Li XF, Zhang S, Gong F, Lu G, Lin G. Increased pregnancy complications following frozen-thawed embryo transfer during an artificial cycle. J Assist Reprod Genet. 2019;36(5):925-33.
22. Makhijani R, Bartels C, Godiwala P, Bartolucci A, Nulsen J, Grow D, et al. Maternal and perinatal outcomes in programmed versus natural vitrified-warmed blastocyst transfer cycles. Reproductive biomedicine online. 2020 Aug;41(2):300-8.
23. Asserhøj LL, Spangmose AL, Aaris Henningsen AK, Clausen TD, Ziebe S, Jensen RB, et al. Adverse obstetric and perinatal outcomes in 1,136 singleton pregnancies conceived after programmed frozen embryo transfer (FET) compared with natural cycle FET. Fertility and sterility. 2021 Apr;115(4):947-56.
24. Zaat TR, Brink AJ, de Bruin JP, Goddijn M, Broekmans FJM, Cohlen BJ, et al. Increased obstetric and neonatal risks in artificial cycles for frozen embryo transfers? Reproductive biomedicine online. 2021 May;42(5):919-29.
25. Berntsen S, Pinborg A. Large for gestational age and macrosomia in singletons born after frozen/thawed embryo transfer (FET) in assisted reproductive technology (ART). Birth defects research. 2018 May 1;110(8):630-43.
26. 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? Human reproduction update. 2018 Jan 1;24(1):35-58.
27. Wennerholm UB, Henningsen AK, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, et al. Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. Human reproduction (Oxford, England). 2013 Sep;28(9):2545-53.
28. Wang Z, Liu H, Song H, Li X, Jiang J, Sheng Y, et al. Increased Risk of Pre-eclampsia After Frozen-Thawed Embryo Transfer in Programming Cycles. Front Med (Lausanne). 2020;7:104-.
29. Patel S, Kilburn B, Imudia A, Armant DR, Skafar DF. Estradiol Elicits Proapoptotic and Antiproliferative Effects in Human Trophoblast Cells. Biology of reproduction. 2015 Sep;93(3):74.
30. Gauster M, Desoye G, Tötsch M, Hiden U. The placenta and gestational diabetes mellitus. Current diabetes reports. 2012 Feb;12(1):16-23.
31. Saito K, Kuwahara A, Ishikawa T, Morisaki N, Miyado M, Miyado K, et al. Endometrial preparation methods for frozen-thawed embryo transfer are associated with altered risks of hypertensive disorders of pregnancy, placenta accreta, and gestational diabetes mellitus. Human reproduction (Oxford, England). 2019 Aug 1;34(8):1567-75.
32. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ. Pre-eclampsia. Lancet (London, England). 2016 Mar 5;387(10022):999-1011.
33. Palomba S, Falbo A, Daolio J, Battaglia FA, La Sala GB. Pregnancy complications in infertile patients with polycystic ovary syndrome: updated evidence. Minerva ginecologica. 2018 Dec;70(6):754-60.
34. Zhang WY, von Versen-Höynck F, Kapphahn KI, Fleischmann RR, Zhao Q, Baker VL. Maternal and neonatal outcomes associated with trophectoderm biopsy. Fertility and sterility. 2019 Aug;112(2):283-90.e2.
35. Zheng W, Yang C, Yang S, Sun S, Mu M, Rao M, et al. Obstetric and neonatal outcomes of pregnancies resulting from preimplantation genetic testing: a systematic review and meta-analysis. Human reproduction update. 2021 Oct 18;27(6):989-1012.

36. Baksh S, Casper A, Christianson MS, Devine K, Doody KJ, Ehrhardt S, et al. Natural vs. programmed cycles for frozen embryo transfer: study protocol for an investigator-initiated, randomized, controlled, multicenter clinical trial. Trials. 2021 Sep 27;22(1):660.

Table I: Maternal characteristics in frozen embryo transfers (FET) by cycle regimen.

	Singleton Deliveries (n = 4636)				Twin Deliveries (n = 544)			
Characteristics	**NC-FET (n = 703)**	**SC-FET (n = 662)**	**HRC-FET (n = 3271)**	***P*-value**	**NC-FET (n = 73)**	**SC-FET (n = 96)**	**HRC-FET (n = 375)**	***P*-value**
Maternal age (years), mean (SD)	35.4 (3.9)	35.5 (3.9)	35.2 (4.0)	0.064	34.6 (3.8)	33.8 (4.0)	34.0 (4.3)	0.516
Recurrent miscarriage >2 (%)	2 (0.3)	2 (0.3)	25 (0.8)	0.257	0	0	1 (0.3)	1.000
Cause of infertility, n (%)								
Chronic anovulation / PCOS	42 (6.0)	70 (10.6)	546 (16.7)	*<0.001*	4 (5.5)	6 (6.3)	84 (22.4)	*<0.001*
Tubal factor	88 (12.5)	107 (16.2)	455 (13.9)	0.147	7 (9.6)	12 (12.5)	45 (12.0)	0.858
Uterine malformation	4 (0.6)	6 (0.9)	34 (1.0)	0.555	1 (1.4)	1 (1.0)	0	0.096
Uterine fibroids	3 (0.4)	13 (2.0)	40 (1.2)	*0.029*	0	2 (2.1)	1 (0.3)	0.131
Endometriosis (I/II)	55 (7.8)	32 (4.8)	244 (7.5)	*0.035*	6 (8.2)	9 (9.4)	21 (5.6)	0.318
Endometriosis (III/IV)	25 (3.6)	27 (4.1)	193 (5.9)	*0.013*	3 (4.1)	2 (2.1)	17 (4.5)	0.626
Hypergonadotropic ovarian insufficiency (WHO III)	12 (1.7)	6 (0.9)	59 (1.8)	0.260	0	0	4 (1.1)	1.000
Hypogonadotropic ovarian insufficiency (WHO I)	1 (0.1)	2 (0.3)	31 (0.95)	*0.029*	0	0	4 (1.1)	1.000
Other female pathologies, n (%)	35 (5.0)	94 (14.2)	345 (10.5)	*<0.001*	3 (4.1)	12 (12.5)	35 (9.3)	0.171
Comorbidities, n (%)								
Diabetes mellitus I/II	1 (0.1)	2 (0.3)	4 (0.1)	0.330	0	0	2 (0.5)	1.000
Thyroid disease	25 (3.6)	24 (3.6)	187 (5.7)	*0.010*	3 (4.1)	2 (2.1)	25 (6.7)	0.214
Breast cancer	3 (0.4)	1 (0.2)	6 (0.2)	0.360	0	0	0	
Malignancy of the genital tract	0	0	7 (0.2)	0.586	0	0	1 (0.3)	1.000
Treatment type, n (%)								
IVF	121 (17.2)	136 (20.5)	538 (16.4)	*<0.001*	9 (12.3)	26 (27.1)	62 (16.5)	*<0.001*
ICSI	547 (77.8)	292 (44.1)	2616 (80.0)		59 (80.8)	30 (31.3)	296 (78.9)	
Mixed	35 (5.0)	234 (35.4)	117 (3.6)		5 (6.8)	40 (41.7)	17 (4.5)	
Number of embryos / zygotes transferred, n (%)								
1	376 (53.5)	286 (43.2)	2015 (61.6)	*<0.001*	7 (9.6)	2 (2.1)	27 (7.2)	0.083
2	315 (44.8)	332 (50.2)	1205 (36.8)		63 (86.3)	85 (88.5)	332 (88.5)	
3	12 (1.7)	44 (6.6)	51 (1.6)		3 (4.1)	9 (9.4)	16 (4.3)	

Table II: Pregnancy outcome of singletons (n = 4636) in frozen embryo transfers (FET) by cycle regimen.

Outcomes	Deliveries (n = 4636)			Multivariate analysis					
	Incidences (%)			HRC-FET vs. NC-FET		HRC-FET vs. SC-FET		SC-FET vs. NC-FET	
Pregnancy pathology (%)	**NC-FET (n = 703)**	**SC-FET (n = 662)**	**HRC-FET (n = 3271)**	**Adjusted OR (95% CI)**	***P* - value**	**Adjusted OR (95% CI)**	***P* - value**	**Adjusted OR (95% CI)**	***P* - value**
Bleeding 1. trimester	20 (2.8)	17 (2.6)	230 (7.0)	2.23 (1.33-3.75)	*0.003*	2.08 (1.03-4.21)	*0.042*	1.07 (0.47-2.45)	0.870
Bleeding 2. trimester	5 (0.7)	6 (0.9)	39 (1.2)	2.09 (0.77-5.69)	0.150	1.42 (0.46-4.40)	0.543	1.47 (0.35-6.11)	0.596
Bleeding 3. trimester	9 (1.3)	6 (0.9)	24 (0.7)	0.55 (0.23-1.30)	0.173	1.18 (0.38-3.65)	0.779	0.46 (0.13-1.61)	0.227
Premature labour 2. trimester	6 (0.9)	1 (0.2)	28 (0.9)	n.a.	-	n.a.	-	n.a.	-
Premature labour 3. trimester	11 (1.6)	2 (0.3)	37 (1.1)	n.a.	-	n.a.	-	n.a.	-
Premature rupture of membranes	10 (1.4)	9 (1.4)	101 (3.1)	1.20 (0.56-2.54)	0.643	1.07 (0.40-2.82)	0.898	1.12 (0.36-3.52)	0.845
Placenta praevia	8 (1.1)	6 (0.9)	32 (1.0)	0.94 (0.40-2.22)	0.888	1.30 (0.43-3.93)	0.647	0.73 (0.20-2.60)	0.622
Isolated hypertension >140/90mmHg	6 (0.9)	1 (0.2)	60 (1.8)	2.50 (1.02-6.12)	*0.045*	1.30 (0.43-3.93)	0.647	0.38 (0.04-3.48)	0.391
Preeclampsia	12 (1.7)	2 (0.3)	93 (2.8)	2.16 (1.13-4.12)	*0.019*	6.02 (1.38-26.24)	*0.017*	0.36 (0.07-1.74)	0.203
Eclampsia	2 (0.3)	9 (1.4)	5 (0.2)	n.a.	-	n.a.	-	n.a.	-
Intrauterine growth restriction (IUGR)	13 (1.8)	1 (0.2)	42 (1.3)	n.a.	-	n.a.	-	n.a.	-
Gestational diabetes	32 (4.6)	46 (6.9)	147 (4.5)	0.96 (0.61-1.52)	0.873	0.51 (0.30-0.88)	*0.016*	1.88 (0.99-3.57)	0.053
Cervical insufficiency with cerclage	1 (0.1)	5 (0.8)	8 (0.2)	1.93 (0.22-17.03)	0.554	0.52 (0.12-2.21)	0.374	3.73 (0.34-41.35)	0.283
Hospitalisation in pregnancy	15 (2.1)	24 (3.6)	97 (3.0)	1.62 (0.88-2.97)	0.119	1.26 (0.65-2.44)	0.497	1.29 (0.57-2.93)	0.545
Cholestasis	1 (0.1)	0 (0)	8 (0.2)	n.a.	-	n.a.	-	n.a.	-
Unknown	0	1 (0.2)	4 (0.1)	n.a.	-	n.a.	-	n.a.	-
Other	337 (47.9)	278 (42.0)	931 (28.5)	0.39 (0.32-0.48)	*<.001*	0.24 (0.18-2.19)	*<.001*	1.60 (1.18-2.19)	*0.003*

Table III: Pregnancy outcome of twins (n = 544) in frozen embryo transfers (FET) by cycle regimen.

Outcomes	Deliveries (n = 544)			Multivariate analysis					
	Incidences (%)			HRC-FET vs. NC-FET		HRC-FET vs. SC-FET		SC-FET vs. NC-FET	
Pregnancy pathology (%)	**NC-FET (n = 703)**	**SC-FET (n = 662)**	**HRC-FET (n = 3271)**	**Adjusted OR (95% CI)**	***P* - value**	**Adjusted OR (95% CI)**	***P* - value**	**Adjusted OR (95% CI)**	***P* - value**
Bleeding 1. trimester	3 (4.1)	1 (1.0)	20 (5.3)	1.62 (0.40-6.50)	0.497	5.52 (0.54-56.43)	0.149	0.29 (0.02-3.89)	0.351
Bleeding 2. trimester	2 (2.7)	1 (1.0)	9 (2.4)	0.92 (0.18-4.61)	0.918	5.92 (0.63-55.81)	0.120	0.16 (0.01-1.99)	0.152
Bleeding 3. trimester	1 (1.4)	3 (3.1)	7 (1.9)	1.39 (0.16-11.80)	0.765	0.97 (0.18-5.16)	0.976	1.42 (0.12-16.40)	0.777
Premature labour 2. trimester	1 (1.4)	0	13 (3.5)	n.a.	-	n.a.	-	n.a.	-
Premature labour 3. trimester	8 (11.0)	4 (4.2)	33 (8.8)	n.a.	-	n.a.	-	n.a.	-
Premature rupture of membranes	4 (5.5)	7 (7.3)	30 (8.0)	1.64 (0.53-5.05)	0.386	0.85 (0.31-2.30)	0.745	1.94 (0.48-7.80)	0.351
Placenta praevia	0 (0)	0 (0)	3 (0.8)	n.a.	-	n.a.	-	n.a.	-
Isolated hypertension >140/90mmHg	0 (0)	0 (0)	7 (1.9)	n.a.	-	n.a.	-	n.a.	-
Preeclampsia	2 (2.7)	1 (1.0)	27 (7.2)	2.54 (0.54-11.94)	0.238	4.05 (0.47-34.74)	0.201	0.63 (0.05-8.18)	0.721
Eclampsia	0 (0)	6 (6.3)	3 (0.8)	n.a.	-	n.a.	-	n.a.	-
Intrauterine growth restriction (IUGR)	6 (8.2)	0 (0)	11 (2.9)	n.a.	-	n.a.	-	n.a.	-
Gestational diabetes	4 (5.5)	5 (5.2)	18 (4.8)	0.89 (0.26-2.99)	0.845	1.31 (0.35-4.87)	0.685	0.68 (0.14-3.35)	0.630
Cervical insufficiency with cerclage	0 (0)	0 (0)	3 (0.8)	n.a.	-	n.a.	-	n.a.	-
Hospitalisation in pregnancy	9 (12.3)	13 (13.5)	48 (12.8)	1.76 (0.73-4.28)	0.208	0.96 (0.37-2.44)	0.925	1.85 (0.56-6.05)	0.311
Cholestasis	1 (1.4)	0 (0)	6 (1.6)	n.a.	-	n.a.	-	n.a.	-
Unknown	0 (0)	0 (0)	2 (0.5)	n.a.	-	n.a.	-	n.a.	-
Other	34 (46.6)	38 (39.6)	136 (36.3)	0.44 (0.24-0.80)	*0.007*	0.52 (0.28-0.95)	*0.033*	0.85 (0.39-1.83)	0.670

Table IV: Neonatal outcome of singletons and twins in frozen embryo transfers (FET) by cycle regimen.

	Singleton Deliveries (n = 4636)				Twin Deliveries (n = 544)			
Neonatal Outcome	**NC-FET (n = 703)**	**SC-FET (n = 662)**	**HRC-FET (n = 3271)**	***P*-value**	**NC-FET (n = 73)**	**SC-FET (n = 96)**	**HRC-FET (n = 375)**	***P*-value**
Gestational age (%)								
Mean, weeks (w) (SD)	38.8 (1.9)	38.6 (3.1)	38.7 (2.3)	0.141	35.8 (2.3)	35.3 (3.2)	35.3 (3.6)	0.552
Postterm > 42 w	3 (0.4)	4 (0.6)	19 (0.6)	0.745	0 (0)	0 (0)	2 (0.5)	0.260
≥ 37 – < 42 w	626 (89.0)	581 (87.8)	2909 (88.9)		27 (37.0)	25 (26.0)	119 (31.7)	
≥ 32 – < 37 w	64 (9.1)	63 (9.5)	291 (8.9)		43 (58.9)	65 (67.7)	214 (57.1)	
≥ 28 – < 32 w	8 (1.1)	6 (0.9)	27 (0.8)		1 (1.4)	2 (2.1)	26 (6.9)	
< 28 w	2 (0.3)	8 (1.2)	25 (0.8)		2 (2.7)	4 (4.2)	14 (3.7)	
Delivery mode (%)								
Spontaneous	360 (51.2)	298 (45.0)	1104 (33.8)	**<0.001**	8 (11.0)	11 (11.5)	51 (13.6)	0.405
Forceps	11 (1.6)	25 (3.8)	69 (2.1)		0 (0)	0 (0)	4 (1.1)	
Vacuum	57 (8.1)	20 (3.0)	388 (11.9)		2 (2.7)	0 (0)	13 (3.5)	
Caesarean section	270 (38.4)	293 (44.3)	1668 (51.0)		63 (86.3)	83 (86.5)	301 (80.3)	
Unknown / Missing	5 (0.7)	26 (3.9)	42 (1.3)		0	2 (2.1)	6 (1.6)	
Neonate 1: Birthweight (%)								
Mean, g (SD)	3324.4 (523.3)	3316.6 (579.2)	3357.4 (559.3)	0.118	2477.5 (523.7)	2437.2 (566.1)	2440.5 (616.9)	0.881
≥ 4000 g	63 (9.0)	66 (10.0)	324 (9.9)	0.680	0 (0)	0 (0)	0 (0)	0.969
≥ 2500 – < 4000 g	602 (85.6)	555 (83.8)	2771 (84.7)		38 (52.1)	52 (54.2)	195 (52.0)	
≥ 1500 – 2500 g	32 (4.6)	28 (4.2)	127 (3.9)		31 (42.5)	37 (38.5)	151 (40.3)	
< 1500 g	6 (0.9)	12 (1.8)	40 (1.2)		4 (5.5)	6 (6.3)	27 (7.2)	
Unknown / Missing (g)	0 (0)	1 (0.2)	9 (0.3)		0 (0)	1 (1)	2 (0.5)	
Normal range, percentile (P)	557 (79.2)	548 (82.8)	2637 (80.6)	0.072	60 (82.2)	75 (78.1)	293 (78.1)	0.837
SGA (< 10. P)	94 (13.4)	60 (9.1)	338 (10.3)		7 (9.6)	12 (12.5)	37 (9.9)	
LGA (> 90. P)	49 (7.0)	50 (7.6)	269 (8.2)		6 (8.2)	7 (7.3)	38 (10.1)	
Unknown / Missing (P)	3 (0.4)	4 (0.6)	27 (0.8)		0 (0)	2 (2.1)	7 (1.9)	
Neonate 2: Birthweight (%)								
Mean, g (SD)	n.a.				2410.3 (535.9)	2388.6 (616.6)	2382.0 (605.1)	0.934
≥ 4000 g					0 (0)	1 (1.0)	0 (0)	0.469
≥ 2500 – < 4000 g					35 (48.0)	43 (44.8)	181 (48.3)	
≥ 1500 – 2500 g					33 (45.2)	44 (45.8)	158 (42.1)	
< 1500 g					5 (6.9)	7 (7.3)	33 (8.8)	
Unknown / Missing (g)					0 (0)	1 (1.0)	3 (0.8)	
Normal range, percentile (P)					57 (78.1)	73 (76.0)	284 (75.7)	0.981
SGA (< 10. P)					11 (15.1)	13 (13.5)	50 (13.3)	
LGA (> 90. P)					5 (6.9)	8 (8.3)	33 (8.8)	
Unknown / Missing (P)					0 (0)	2 (2.1)	8 (2.1)	