RBMO

ARTICLE

Increased obstetric and neonatal risks in artificial cycles for frozen embryo transfers?

BIOGRAPHY

Tjitske Zaat is a PhD student and fertility doctor at the Amsterdam University Medical Center. The focus of her PhD is frozen embryo transfer, the freeze-all strategy, and the obstetrical and neonatal outcomes after different types of endometrial preparation in frozen embryo transfers.

Tjitske R. Zaat[1], Anna J. Brink[1], Jan-Peter de Bruin[2], Mariëtte Goddijn[1], Frank J.M. Broekmans[3], Ben J. Cohlen[4], Nick S. Macklon[5], Madelon van Wely[1], Eva R. Groenewoud[6], Femke Mol[1,*] ANTARCTICA trial study group

KEY MESSAGE

Women conceiving by modified NC-FET have a decreased risk of hypertensive disorders of pregnancy compared to AC-FET. This lower risk, combined with the same efficacy compared to AC-FET, can be interpreted as modified NC-FET being the preferred treatment in women with ovulatory cycles undergoing FET. Whether modified NC-FET is the preferred treatment when compared with other endometrium preparation methods aside from AC-FET should be further investigated in future RCT.

ABSTRACT

Research question: What are the obstetric and neonatal risks for women conceiving via frozen–thawed embryo transfer (FET) during a modified natural cycle compared with an artificial cycle method.

Design: A follow-up study to the ANTARCTICA randomized controlled trial (RCT) (NTR 1586) conducted in the Netherlands, which showed that modified natural cycle FET (NC-FET) was non-inferior to artificial cycle FET (AC-FET) in terms of live birth rates. The current study collected data on obstetric and neonatal outcomes of 98 women who had a singleton live birth. The main outcome was birthweight; additional outcomes included hypertensive disorder of pregnancy, premature birth, gestational diabetes, obstetric haemorrhage and neonatal outcomes including Apgar scores and admission to the neonatal ward or the neonatal intensive care unit and congenital anomalies.

Results: Data from 82 out of 98 women were analysed according to the per protocol principle. There was no significant difference in the birthweights of children born between groups (mean difference –124 g [–363 g to 114 g]; $P = 0.30$). Women who conceived by modified NC-FET have a decreased risk of hypertensive disorders of pregnancy compared with AC-FET (relative risk 0.27; 95% CI 0.08–0.94; $P = 0.031$). Other outcomes, such as rates of premature birth, gestational diabetes or obstetric haemorrhage and neonatal outcomes, were not significantly different.

Conclusions: The interpretation is that modified NC-FET is the preferred treatment in women with ovulatory cycles undergoing FET when the increased risk of obstetrical complications and potential neonatal complications in AC-FET are considered.

[1] Amsterdam UMC, University of Amsterdam, Center for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Meibergdreef 9 Amsterdam, the Netherlands
[2] Department of Obstetrics and Gynecology, Jeroen Bosch Ziekenhuis, Henri Dunantstraat 1, 's-Hertogenbosch, the Netherlands
[3] Department for Reproductive Medicine, University Medical Centre Utrecht, Heidelberglaan 100 Utrecht, the Netherlands
[4] Isala Fertility Centre, Isala Clinics, Dokter van Heesweg 2, Zwolle, the Netherlands
[5] Department of Obstetrics and Gynecology, Academic Unit of Human Development and Health, University of Southampton, University Road, Southampton, UK
[6] Department of Obstetrics and Gynaecology, Noordwest Ziekenhuisgroep, Huisduinerweg 3, Den Helder, the Netherlands

*Corresponding author. E-mail address: f.mol@amsterdamumc.nl (F. Mol). https://doi.org/10.1016/j.rbmo.2021.01.015 1472-6483/

KEYWORDS

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Natural cycle
Safety

INTRODUCTION

It has been more than 30 years since the first successful frozen embryo transfer (FET) (*Trounson and Mohr, 1983*; *Zeilmaker et al., 1984*), and it is now increasingly used throughout the world (*De Geyter et al., 2018*; *ESHRE, 2018*; *Pereira et al., 2019*). For FET to be effective, the endometrium needs to be synchronized with the developmental stage of the embryo to allow implantation. The two most commonly used methods to prepare the endometrium and optimize timing of FET are the artificial cycle (AC-FET) using exogenous oestrogen administration to develop the endometrium and subsequent administration of exogenous progesterone to prepare the endometrium and time FET; or the natural cycle (NC-FET), with a natural build-up of the endometrium while using detection of the LH surge to time the embryo transfer. A variant of NC-FET is modified NC-FET, in which the dominant follicle is monitored by repeated ultrasounds followed by human chorionic gonadotrophin injection to trigger ovulation (*Glujovsky et al., 2010*).

In 2017 a Cochrane review, specifically focused on endometrium preparation for FET, concluded that no one type of endometrium preparation in FET was to be preferred over another in subfertile women with regular ovulatory cycles (*Ghobara et al., 2017*). Aside from the chances of pregnancy, the safety of mothers and babies after assisted reproduction should also be taken into account.

Initially, before cryopreservation of embryos became common practice, artificial endometrium preparation was only applied for oocyte donation cycles. In these cycles, the recipient woman had no natural endometrium build-up due to premature ovarian insufficiency (POI), which required donation of oocytes and a programmed cycle (*Buster et al., 1983*). As FET became possible due to improved laboratory procedures, the use of artificial endometrium preparation was extended beyond anovulatory women with POI.

Concerning the obstetric and neonatal outcomes after fresh embryo transfer compared with FET, a systematic review and meta-analysis of observational and randomized studies showed that the risk of preterm birth, small for gestational age and low birthweight were reduced after FET compared with fresh transfer. On the other hand, there was a higher risk of large for gestational age babies and hypertensive disorders of pregnancies in FET compared with fresh embryo transfer (*Maheshwari et al., 2018*). These findings could be explained by the extended culture in cryopreservation compared with fresh; freezing and thawing procedures in FET; or the hormonal stimulation of the ovaries in fresh IVF compared with endometrium preparation in FET. The endometrium preparation methods in FET were not specifically reported in the majority of the included studies. Recently, several cohort studies comparing NC-FET to AC-FET from Japan (*Saito et al., 2017*, *2019*), Sweden (*Ernstad et al., 2019*), the USA (*von Versen-Höynck et al., 2019a*) and China (*Wang et al., 2020*) reported on several safety aspects of different methods of endometrium preparation in FET cycles and found fewer complications after NC-FET compared with AC-FET. Even though these studies provide adequate power to investigate relevant associations, their observational study design renders them more prone to confounding and risk of bias. It is possible that, for example, an intrinsic factor has driven the treatment decision or freezing method, which may influence the outcome, other than method of endometrium preparation. Granted, confounding and bias can be controlled for to a certain extent during analysis, but a randomized design remains the gold standard for investigating causal associations (*Fletcher, 2019*). As more is understood about the adverse health outcomes in children conceived through ART and in this case FET, can the potential risks of artificial endometrium preparation be justified for the more liberal indication of ovulatory women?

Whether NC-FET provides better safety prospects than AC-FET remains unclear because data from RCT are lacking. In view of this uncertainty, this study aimed to compare the procedures with respect to safety prospects in a follow-up study of the ANTARCTICA RCT (*Groenewoud et al., 2016*). It is postulated that modified NC-FET results in fewer babies with a higher birthweight, fewer hypertensive disorders of pregnancy, less ante-partum haemorrhage (including placental pathology) and more gestational diabetes mellitus compared with AC-FET.

MATERIALS AND METHODS

Participants

This study is a follow-up study to the multicentre ANTARCTICA RCT. A total of 1032 women undergoing IVF or intracytoplasmic sperm injection (ICSI) were included for one FET cycle. Inclusion criteria were (i) age between 18 and 40 years; (ii) first, second or third IVF or IVF/ICSI cycle; (iii) regular menstrual cycle. Live birth rate (LBR) after modified NC-FET was 11.5% (57/495) versus 8.8% in AC-FET (41/464), resulting in a relative difference in LBR of –0.027 in favour of modified NC-FET (95% CI –0.065 to 0.012; $P = 0.171$). In the modified NC-FET group, one triplet and two twins were born, while in the AC-FET group one twin was born.

Further details about the ANTARCTICA RCT have been published elsewhere (*Groenewoud et al., 2012*, *2016*).
This study population comprised all women with live births ($n = 98$) in the ANTARCTICA RCT, accomplished after randomized assignment to either modified NC-FET (57 women with live birth) or AC-FET (41 women with live birth). The median age of the children at time of follow-up was 7.62 years (range 4.93–10.14 years). The follow-up study investigated outcomes concerning the first month post-partum of all children.

Outcomes

Birthweight was chosen as the main outcome and this is reported as: absolute birthweight (g); relative birthweight as expressed in percentiles (*Hoftiezer et al., 2019*); large for gestational age (LGA >90th percentile); and small for gestational age (SGA <10th percentile).

Additional outcomes included the following.

Obstetric outcomes

Crown–rump length (CRL) at 8+0 to 12+6 week of gestation [plotted on a Dutch reference curve based on data from the Dutch population (Astraia Software GmbH, Munich, Germany)] (*Hoftiezer et al., 2019*); in case more than one CRL measurement was performed for one woman, the measurement that was closest to 10+0 weeks of gestation was used for analyses (*Koster et al., 2008*); hypertensive disorders of pregnancy (comprising pregnancy-induced hypertension [PIH], pre-eclampsia and haemolysis, elevated liver enzymes, and low platelets in the

blood [HELLP syndrome)]; gestational diabetes; ante-partum haemorrhage (comprising bleeding due to placental abruption); placenta previa; placenta accrete; preterm birth (iatrogenic versus spontaneous); mode of delivery; the occurrence of shoulder dystocia; post-partum haemorrhage (blood loss ≥1000 ml) and retained placenta.

Neonatal outcomes

Apgar score <7 at 5 min after birth; admission to the neonatal ward (duration and reason); admission to the neonatal intensive care unit; congenital anomalies and child mortality (defined as death of the child before the age of 5 years).

Data handling

Data were retrieved by the participating hospitals from their electronic data files. If the data were incomplete, participants were asked to fill out a web-based questionnaire via a web-based service (Castor Electronic Data Capture, Ciwit BV, Amsterdam, the Netherlands) or on paper (Supplementary Information 1). Data were collected on a web-based case record form (Castor Electronic Data Capture, 2016). Data handling was performed with a coded set, with the participant code only available to members of the study group and research nurses at the participating hospitals.

Statistical analysis

Baseline characteristics of all participating women in the ANTARCTICA RCT did not show any significant differences between the study groups (*Groenewoud et al., 2016*). Because a subset with live birth was selected in the present study, the comparison of the baseline characteristics was repeated. All outcomes were analysed according to the per protocol principle. Multiple pregnancies were excluded from the statistical analysis (modified NC-FET $n = 3$; AC-FET $n = 1$) but outcomes are provided. Continuous variables are expressed as mean with SD and categorical variables are expressed as number and percentage of the total allocation arm per live birth. *P*-values below 0.05 are considered to be statistically significant differences. All analyses were performed using SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA).

Ethical considerations

This study was designed using good clinical practice guidelines. The ANTARCTICA trial was registered on the Netherlands trial register as number NTR 1586 (trial registration date: 23 July 2017), approved by the Medical Ethics Committee (MEC) of the Isala Clinics in Zwolle (2009) and by the institutional review boards of the participating centres. During the RCT, written informed consent was obtained from the patient as well as the partner, including permission to investigate obstetric and neonatal outcomes during a later phase. The MEC of the Academic Medical Center (AMC) reviewed and approved the questionnaires and protocol for the study and provided a non-WMO statement on 26 September 2019 (MEC no. 2009_115). Due to European Union privacy regulations (General Data Protection Regulation, May 2018), the privacy officer of Amsterdam UMC, location AMC, advised that renewed informed consents needed to be obtained in all women with a live birth.

The method for follow-up to obtain renewed informed consent was to first approach the women by telephone. After consent by phone, women received the informed consent and questionnaire by post and returned the completed documents to the study group. If the documents were not returned within 6 weeks, women were approached a second time by telephone, as a reminder. When necessary a total of three reminders were performed via telephone, post or email.

RESULTS

A total of 98 women were reported to have a live birth following the ANTARCTICA RCT.

Two women in the modified NC-FET group were excluded from follow-up, one woman because of a lack of contact data and one because she appeared not to have a live birth following the ANTARCTICA RCT. Of the 96 women who were contacted for follow-up, 10 women declined to provide informed consent for collection of the data. Informed consent was received from 82 women, resulting in a follow-up rate of 85.4% (82/96 women). Women with multiple pregnancy were excluded from the analysis (modified NC-FET $n = 3$; AC-FET $n = 1$) (cases reported in Supplementary Information 2). Reported here are the results of the per protocol analysis of 82 women with live birth in the ANTARCTICA RCT (modified NC-FET $n = 45$; AC-FET $n = 37$) (FIGURE 1). Baseline was comparable between groups of the 98 women with live birth in the ANTARCTICA RCT (modified NC-FET $n = 57$, AC-FET $n = 41$) (TABLE 1). Baseline characteristics were comparable in the analysed cohort.

Main outcome

The mean birthweight was 3610 g (±580 g) for children born from modified NC-FET compared with 3735 g (±487 g) for children born from AC-FET (mean difference –124 g [95% CI –363 to 114 g]) (TABLE 2). No differences between modified NC-FET and AC-FET were found for mean birthweight percentile, SGA and LGA rates (TABLE 2). Data comparing this study group with the healthy low-risk Dutch population are plotted on the reference curves for the birthweight percentiles adjusted for gestational age and gender (FIGURE 2). Compared to the healthy Dutch population it was found that after any FET cycle the mean birth percentile (68th percentile for modified NC-FET and 67th percentile for AC-FET) was relatively high but not significantly different between groups ($P = 0.30$, mean difference –124 [95% CI –363 to 114]) or compared with the healthy Dutch population (mean percentile difference –0.85 [95% CI –13.3 to 11.6]) (TABLE 2).

Additional obstetric outcomes

Data on CRL measurements were available for 55.6% (25/45) in the modified NC-FET group and 67.6% (25/37) in the AC-FET group. The CRL measurements are shown in FIGURE 3. Except for one measurement in the AC-FET group, all measurements for both the modified NC-FET group and the AC-FET are above the mean and the majority of the measurements are above +1 SD of the Dutch reference group. The percentile of CRL is plotted with the percentile of the birthweight per live birth in FIGURE 4. The majority of children in both groups are in the upper right quadrant, i.e. a relatively large CRL in early pregnancy (FIGURE 4).

The obstetric outcomes are reported in TABLE 3. Women undergoing modified NC-FET have a decreased risk of developing hypertensive disorders of pregnancy compared with women undergoing AC-FET (relative risk [RR] 0.27; 95% CI 0.08–0.94; $P = 0.031$). Other obstetric outcomes such as rates of premature

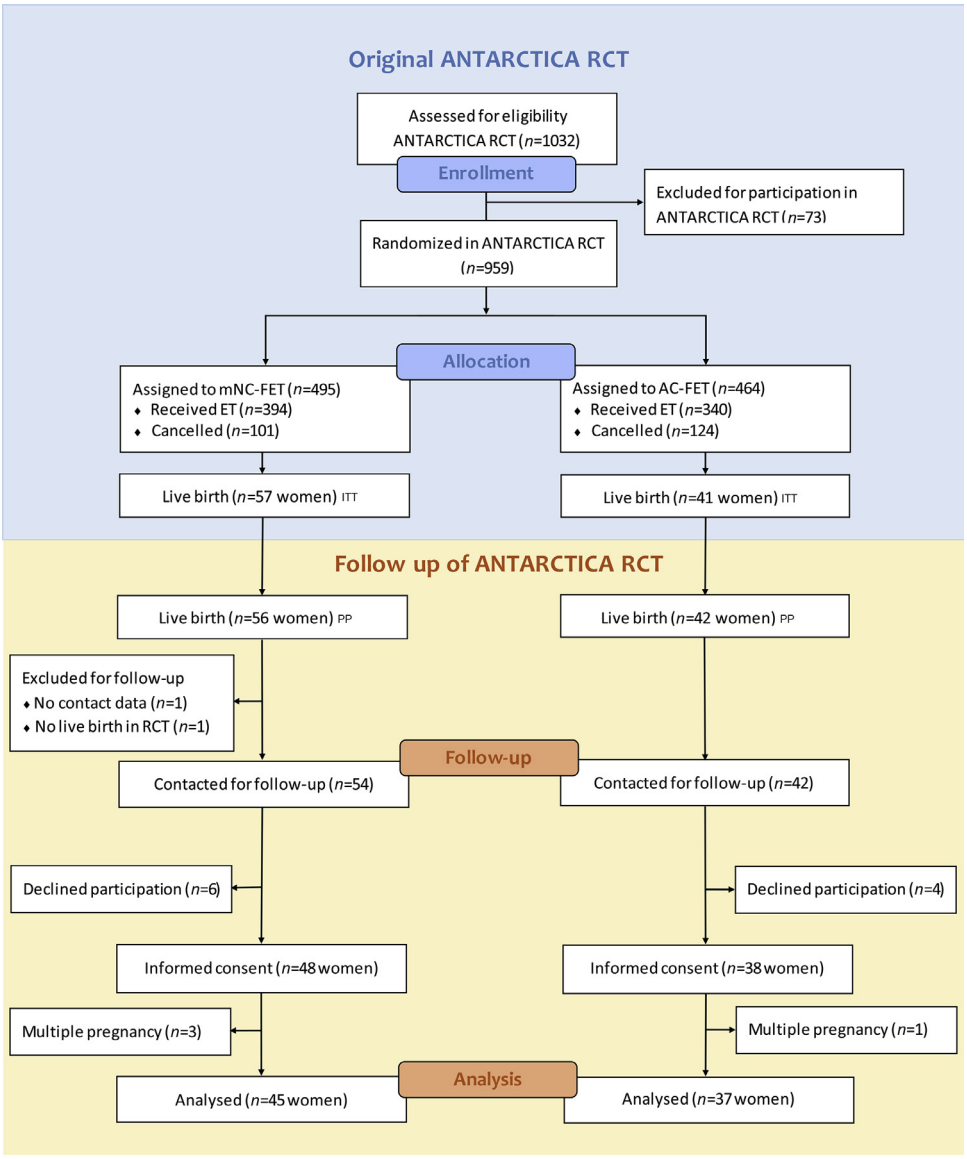

FIGURE 1 Flow diagram of the ANTARCTICA RCT and follow-up study. *n* increases between ITT and PP in the AC-FET group (i.e. 41 to 42) and decreases in the modified NC-FET group (i.e. 57 to 56) because one woman switched from modified NC-FET to AC-FET after randomization. AC-FET = artificial cycle frozen–thawed embryo transfer; ET = embryo transfer; ITT = intention-to-treat analysis, mNC-FET = modified natural cycle frozen–thawed embryo transfer; NC-FET = natural cycle frozen–thawed embryo transfer; PP = per protocol analysis; RCT = randomized controlled trial.

birth, gestational diabetes or obstetric haemorrhage did not differ between groups (TABLE 3).

Neonatal outcomes, including reason for neonatal ward admission and congenital anomalies according to the ICD-10 classification (*WHO, 2010*), are shown in TABLE 4. Neonatal outcomes did not differ between groups. All children were alive at the time of follow-up (TABLE 4).

Outcomes of multiple pregnancies

Three multiple pregnancies occurred in the modified NC-FET group and one in the AC-FET group. No congenital anomalies or deaths occurred. The outcomes are summarized in Supplementary Information 2.

DISCUSSION

Birthweights of children born after modified NC-FET compared with AC-FET in the ANTARCTICA RCT are not significantly different. Children born after modified NC-FET and AC-FET show an increased birthweight compared with the healthy Dutch population, but this did not reach statistical significance. In this study, women who conceived through modified NC-FET had a decreased risk of developing hypertensive disorders of pregnancy compared with women conceived through AC-FET.

Previous research tells us that babies born from FET have a higher mean birthweight and are more likely to be LGA compared with babies born from fresh embryo transfer (*Berntsen and Pinborg, 2018*; *Maheshwari et al., 2018*; *Wennerholm et al., 2013*). The higher risk of having a LGA baby after FET is also applicable when compared with the

TABLE 1 BASELINE CHARACTERISTICS

	Follow-up of ANTARCTICA RCT (ITT[a])			Original ANTARCTICA RCT (ITT[a])	
	Modified NC-FET	**AC-FET**	***P*-value**	**Modified NC-FET**	**AC-FET**
Treatment allocation				495 (51.6)	464 (48.4)
Women who received ET				394 (79.6)	340 (73.3)
Live birth	57 (100)	41 (100)		57 (14.5)	41 (12.1)
Age at treatment (years)	32.3 ± 4.3	33.7 ± 3.9	0.10	33.3 ± 4.0	33.8 ± 4.0
Duration of infertility (years)	2.59 ± 2.1	3.07 ± 2.8	0.35	2.9 ± 2.2	3.1 ± 2.5
Previous IVF cycle	35 (61.4)	26 (63.4)	1.00	221 (44.6)	217 (46.8)
Previous pregnancy	39 (68.4)	26 (63.4)	0.67	299 (60.4)	273 (58.9)
Parity ≥1	25 (43.9)	21 (51.2)	0.35	211 (42.6)	193 (42.0)
Cause of infertility**					
Unknown	3 (5.3)	0 (0.0)	NA	113 (22.8)	98 (21.1)
Male factor	30 (52.6)	27 (65.9)	0.22	271 (54.7)	282 (60.8)
Tubal factor	8 (14.0)	6 (14.6)	1.00	79 (15.9)	61 (13.1)
Hormonal factor	1 (1.8)	0 (0)	NA	20 (4.0)	9 (1.9)
Endometriosis	4 (7.0)	1 (2.4)	0.40	36 (7.3)	28 (6.0)
Unexplained/other	11 (19.3)	7 (17.1)	0.82	11 (2.2)	15 (3.2)
ICSI treatment	29 (50.9)	24 (58.5)	0.54	264 (53.3)	296 (63.8)
Stage at ET			0.64		
Cleavage stage (day 3 or 4)	54 (94.7)	40 (97.5)		454 (91.7)	432 (93.1)
Blastocyst stage (day 5)	3 (5.3)	1 (2.5)		41 (8.3)	32 (6.9)
No. of embryos transferred			1.00	1.0 ± 0.58	0.96 ± 0.61
1	40 (70.1)	29 (70.7)			
≥2	17 (29.8)	12 (29.3)			

Data are presented as *n* (%) or mean ± SD.

AC-FET = artificial cycle frozen–thawed embryo transfer; ET = embryo transfer; ITT = intention-to-treat; NC-FET = natural cycle frozen–thawed embryo transfer; RCT = randomized controlled trial.

[a] ITT: data presented as intention-to-treat for baseline characteristics. As shown in **FIGURE 1**, one woman assigned to the modified NC-FET group eventually received AC-FET in the ANTARCTICA RCT.

general population (*Luke et al., 2017*; *Pinborg et al., 2014*; *Spijkers et al., 2017*). Using an AC-FET cycle as a method of endometrial preparation has been suggested as a possible confounder for macrosomia after FET (*Ernstad et al., 2019*). The findings of this study did not show a statistically significant difference between FET protocols, although the mean birthweight was relatively high in both groups (but not statistically different) compared with the healthy Dutch population (https://www.cbs.nl/nl-nl/cijfers/detail/37302). The percentage of LGA was also high for both FET protocols, which is in line with previously published studies (*Berntsen and Pinborg, 2018*; *Maheshwari et al., 2018*; *Wennerholm et al., 2013*). However, the percentage of LGA did not differ between the study groups. The difference between the outcomes in this study and the previously published large cohort study by *Ernstad et al. (2019)* may be explained by the small sample size here, but also by a difference in baseline of the study participants. Women participating in the ANTARCTICA RCT all had ovulatory cycles, in contrast to women included in the cohort study by *Ernstad et al. (2019)*, where a large percentage, especially in the AC-FET group, had polycystic ovary syndrome.

Assuming that higher birthweights in babies born from FET could be a result of different endometrium preparation methods, difference in antenatal growth could provide more information about the biological explanation. The current literature about antenatal growth in relation to birthweight after FET is limited. In 2014, *Eindhoven et al. (2014)* were the first to research first-trimester measurements after IVF/ICSI treatment, where they found no significant difference between embryonic and fetal growth trajectories and birthweight between pregnancies conceived with IVF/ICSI treatment and naturally conceived pregnancies. *Von Versen-Höynck et al. (2018)* reported on a significantly smaller CRL at 6 weeks' gestation for conceptions achieved via fresh embryo transfer compared with FET in a natural cycle. In the current study, almost all of the CRL measurements are above the mean, which is in line with the study by *Versen-Höynck et al. (2018)* and most of the data are even above the mean + 1SD (**FIGURE 3**). Looking at the relationship between CRL and birthweight it was found that the majority of the live births had both CRL and birthweight above the 50th percentile (**FIGURE 4**). This perhaps implies that neonatal growth is already increased in the first trimester in this study group, in both modified NC-FET and AC-FET.

TABLE 2 MAIN OUTCOME: BIRTHWEIGHT[a] (PP[b])

	Modified NC-FET	AC-FET	*P*-value	
Live birth	45 (100)	37 (100)		Mean difference (95% CI)
Birthweight (g)	3610 ± 580	3735 ± 487	0.30	–124 (–363, 114)
Gestational age (weeks)	39.0 ± 1.80	39.4 ± 1.07	0.10	–3.88 (–8.56, 0.80)
Birthweight category				RR (95% CI)
≥4500 g (macrosomia)	2 (4.4)	2 (5.4)	1.00	0.82 (0.12, 5.56)
≥4000 g (macrosomia)	8 (17.8)	11 (29.7)	0.29	0.60 (0.27, 1.33)
2500–4000 g	33 (73.3)	24 (64.9)	0.47	1.13 (0.84, 1.52)
<2500 g	2 (4.4)	0 (0)	0.50	NA
<1500 g	0 (0)	0 (0)	NA	NA
Birthweight based on Hoftiezer[c]				Mean percentile difference (95% CI)
Birthweight percentile	68 ± 27.9	67 ± 28.5		–0.85 (–13.3, 11.6)
Male	24 (53.3)	19 (51.4)		
Birthweight percentile	67 ± 27.9	69 ± 26.7		
Female	21 (46.7)	18 (48.6)		
Birthweight percentile	69 ± 28.5	66 ± 31.0		
Birthweight percentile category				RR (95% CI)
Large for gestational age (i.e. >90th percentile)	13 (28.9)	11 (29.7)	1.00	0.97 (0.49, 1.91)
Normal for gestational age (i.e. 10th–90th percentile)	29 (64.4)	25 (67.6)	0.82	0.95 (0.70, 1.30)
Small for gestational age (i.e. <10th percentile)	3 (6.7)	1 (2.7)	0.62	2.47 (0.27, 22.73)

Data are presented as *n* (%) or mean ± SD.

For AC-FET group, data concerning birthweight were derived from the medical record in 97.3% (36/37) and 2.7% (1/37) was patient-reported.

AC-FET = artificial cycle frozen–thawed embryo transfer; ITT = intention-to-treat; NA = not applicable; NC-FET = natural cycle frozen–thawed embryo transfer; RR = relative risk.

[a] Data concerning birthweight were derived for the modified NC-FET group from the medical record in 97.8% (44/45) and 2.2% (1/45) was patient-reported.

[b] PP: per protocol analysis. As shown in **FIGURE 1**, one woman assigned to the modified NC-FET group eventually received AC-FET in the ANTARCTICA RCT. Therefore the analysis is presented as per protocol.

[c] Percentiles based on Dutch reference curve (*Hoftiezer et al., 2019*).

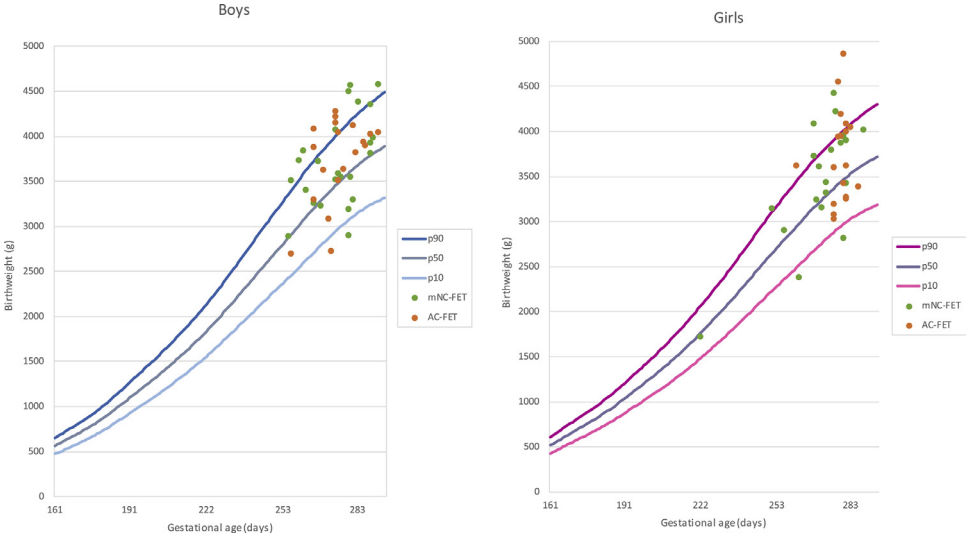

FIGURE 2 Birthweight plotted against the gestational age for modified NC-FET and AC-FET in Dutch normality curves. AC-FET = artificial cycle frozen–thawed embryo transfer; mNC-FET = modified natural cycle frozen–thawed embryo transfer.

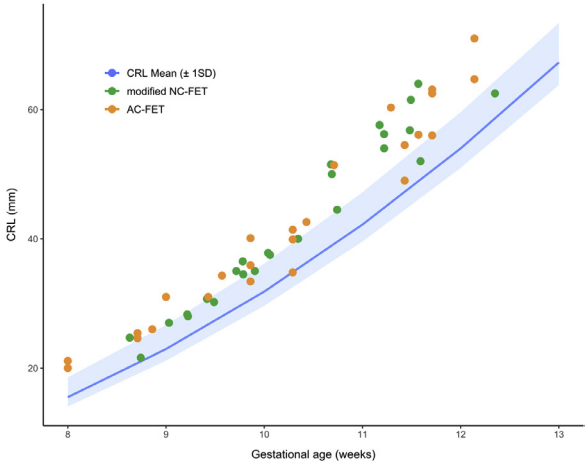

FIGURE 3 Crown–rump length (CRL) of ANTARCTICA children on the reference curve. AC-FET = artificial cycle frozen–thawed embryo transfer; NC-FET = natural cycle frozen–thawed embryo transfer.

Results of this study show that women in the modified NC-FET group have a decreased risk of developing hypertensive disorders of pregnancy compared with women in the AC-FET group, which is in line with previous research (*Saito et al., 2019*; *von Versen-Höynck et al., 2019a*). It is postulated that exogenous oestrogen and progesterone used during AC-FET may cause changes in the endometrial condition and subsequent placental development. Progesterone induces decidualization of the endometrial stromal cells and regulates extravillous trophoblast invasion. Aberrant progesterone levels in early pregnancy may therefore lead to over-invasion of the extravillous trophoblast (*Chen et al., 2012*). *von Versen-Hoynck et al., 2019b* reported on another possible biological explanation: an increased risk of pre-eclampsia with FET because of the absence of a corpus luteum, which is the case during AC-FET. Their results showed significantly more pre-eclampsia in women undergoing FET without a corpus luteum, but no significant difference in the frequency of pre-eclampsia between modified NC-FET and spontaneous conception. In parallel, they found impaired gestational increases of central arterial compliance in the absence of a corpus luteum. It is postulated that the increased risk of pre-eclampsia may be due to missing circulating corpus luteum vasoactive products such as relaxin, vascular endothelial growth factor and angiogenic metabolites of oestrogen. The absence of these vasoactive factors may lead to deficient circulatory adaptations during early gestation and therefore pre-eclampsia (*Conrad, 2011*; *Singh et al., 2020*; *von Versen-Höynck et al., 2018*, 2019).

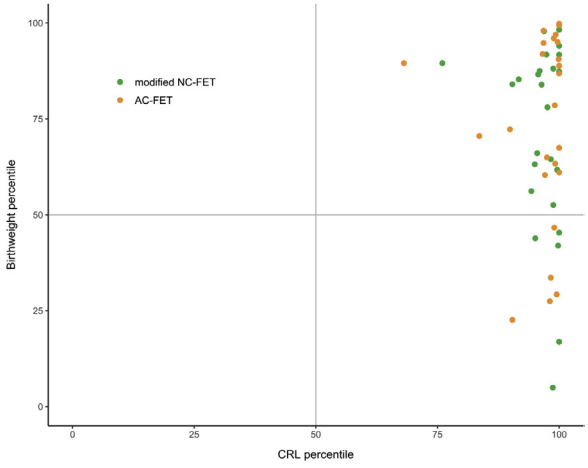

FIGURE 4 CRL percentile versus birthweight percentile. AC-FET = artificial cycle frozen–thawed embryo transfer; CRL = crown–rump length; NC-FET = natural cycle frozen–thawed embryo transfer.

Several studies report on higher rates of placental pathology when FET is compared with fresh embryo transfer (*Ishihara et al., 2014*; *Kaser et al., 2015*; *Sacha et al., 2019*). *Saito et al. (2019)* recently showed that women undergoing AC-FET had an increased risk of placental pathology. Due to the low prevalence of placental pathology and this study's small sample size, no firm conclusions on the effects of modified NC-FET and ante-partum haemorrhage or placental pathology can be drawn.

Although previous studies reported that modified NC-FET results in an increased risk of gestational diabetes mellitus (*Saito et al., 2017*, *2019*), no difference in the incidence of gestational diabetes mellitus between groups was observed in this study.

Results of this study also conflict with the previously reported increased risk of post-term delivery in AC-FET (*Ernstad et al., 2019*; *Saito et al., 2017*). No child was delivered after a gestational age of more than 42 weeks in the current study, and a total of five women were induced for labour because of approaching post-term (modified NC-FET n = 2; AC-FET n = 3).

A major strength of this study is that the follow-up data are from a previously performed RCT. Due to the randomized design, the influence of other possible confounders on these outcomes is probably negligible.

Several uncertainties should be acknowledged. This study has statistical limitations because of its small sample size. Based on a *post hoc* power calculation, with a sample size of 80 and a common SD of 525 g, the study would have been able to detect a mean difference of 340 g (80% power and 5% significance level). Another limitation is based on the large inclusion period: the ANTARCTICA trial took place from 2009 to 2014. Because the main RCT was performed 6 to 11 years ago, definitions differ between hospitals, for example hypertensive disorders of pregnancy, which may have led to heterogeneity. The vast majority of data were collected from hospital files, however the use of data collected from questionnaires – even a small amount – should be acknowledged as a limitation to this study. Given the retrospective data collection there is a risk of recall and selection bias.

TABLE 3 OBSTETRIC OUTCOMES (PP[a])

	Modified NC-FET	AC-FET	*P*-value	RR (95% CI)
Women with a singleton live birth	45 (100)	37 (100)		
Hypertensive disorder of pregnancy[b]	3 (6.7)	9 (24.3)	0.031	0.27 (0.08, 0.94)
PIH	2 (4.4)	6 (16.2)	0.13	0.27 (0.06, 1.28)
PE	1 (2.2)	3 (8.1)	0.32	0.27 (0.03, 2.53)
HELLP	0 (0)	0 (0)	NA	NA
Gestational diabetes mellitus	1 (2.2)	1 (2.7)	1.00	0.82 (0.05, 12.7)
Ante-partum haemorrhage	2 (4.4)	2 (5.4)	1.00	0.82 (0.12, 5.56)
Placenta abruption	1 (2.2)	0 (0)	NA	NA
Placenta previa	2 (4.4)	1 (2.7)	1.00	1.64 (0.16, 17.43)
Placenta accreta	0 (0)	0 (0)	NA	NA
Prematurity				
Iatrogenic preterm birth at <32 weeks	0 (0)	0 (0)	NA	NA
Spontaneous preterm birth at <32 weeks	1 (2.2)	0 (0)	NA	NA
Iatrogenic preterm birth at 32 to <37 weeks	2 (4.4)	1 (2.7)	1.00	1.64 (0.16, 17.43)
Spontaneous preterm birth at 32 to <37 weeks	2 (4.4)	0 (0)	NA	NA
Mode of delivery				
Spontaneous delivery	30 (66.7)	20 (54.1)	0.26	1.23 (0.86, 1.77)
Instrumental delivery	4 (8.9)	5 (13.5)	0.73	0.66 (0.19, 2.28)
Primary caesarean	2 (4.4)	3 (8.1)	0.65	0.55 (0.97, 3.11)
Secondary caesarean	9 (20.0)	9 (24.3)	0.79	0.82 (0.36, 1.86)
Suspected fetal distress	3 (6.7)	0 (0)	NA	NA
Non-progressive labour	4 (8.9)	7 (18.9)	NA	NA
Shoulder dystocia	2 (4.4)	1 (2.7)	1.00	1.64 (0.16, 17.43)
Post-partum haemorrhage, ≥1000 ml	6 (13.3)	10 (27.0)	0.16	0.49 (0.20, 1.23)
Retained placenta	2 (4.4)	5 (13.5)	0.24	0.33 (0.07, 1.60)

Data are presented as *n* (%).

AC-FET = artificial cycle frozen–thawed embryo transfer; NA = not available; NC-FET = natural cycle frozen–thawed embryo transfer; NICU = neonatal intensive care unit; RR = relative risk.

[a] At the time of follow-up all children were alive.

[b] PP: per protocol analysis. As shown in **FIGURE 1** one woman assigned to the modified NC-FET group eventually received AC-FET in the ANTARCTICA RCT. Therefore the analysis is presented as per protocol.

[c]There was one admission to the NICU after neonatal resuscitation following the placental abruption during premature delivery.

Moreover, body mass index and pre-existing hypertensive disorders were not collected in the original data set but because of the randomized design, the influence of these possible confounders is thought to be negligible.

Concerning implications for future research, the development and use of a core outcome set for obstetric and neonatal outcomes in fertility care is needed (*Duffy et al., 2017*, *2018*). A standardized set of outcomes across studies, possibly even merging a paediatric core outcome set with a fertility core outcome set, would facilitate evidence synthesis in meta-analyses and systematic reviews.

In conclusion, birthweights of children born were not significantly different between FET protocols, although the mean birthweight was relatively high in both groups (but not statistically different) compared with the healthy Dutch population. Women undergoing NC-FET have a decreased risk of hypertensive disorders of pregnancy compared with women undergoing AC-FET. In combination with comparable ongoing pregnancy rates and live birth rates of modified NC-FET compared with AC-FET, the interpretation is that modified NC-FET is the preferred treatment in women with ovulatory cycles undergoing FET when the risks of obstetrical complications and potential neonatal complications are considered. Whether modified NC-FET is the preferred treatment when compared with other endometrium preparation methods aside from AC-FET should be further investigated in future RCT. The association between the endometrium preparation method for FET and obstetrical and neonatal complications merits further attention and anticipation during clinical practice in order to optimize the health of both mothers and children after FET.

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TABLE 4 NEONATAL OUTCOMES[a] (PP[b])

	Modified NC-FET	AC-FET	*P*-value	RR (95% CI)
Live birth	45 (100)	37 (100)		
Gender of baby				
Male	24 (53.3)	19 (51.4)	1.00	1.04 (0.69, 1.57)
Female	21 (46.7)	18 (48.6)	1.00	0.96 (0.61, 1.51)
Apgar score <7 at 5 min	1 (2.2)	0 (0)	NA	NA
Admission to neonatal ward	11 (24.4)	3 (8.1)	0.08	3.02 (0.91, 10.01)
Weight loss	1 (2.2)	0 (0)	NA	NA
Wet lung syndrome	1 (2.2)	0 (0)	NA	NA
Prematurity	2 (4.4)	0 (0)	NA	NA
Meconium-stained amniotic fluid and fever	1 (2.2)	1 (2.7)	1.00	0.82 (0.05, 12.7)
Pneumonia after amniotic fluid aspiration	1 (2.2)	0 (0)	NA	NA
Observation due to pre-partum rupture of membranes	3 (6.7)	1 (2.7)	0.43	2.47 (0.27, 22.7)
Observation due to cardiac arrhythmia during pregnancy	1 (2.2)	0 (0)	NA	NA
Observation due to difficult vacuum delivery	1 (2.2)	1 (2.7)	1.00	0.82 (0.05, 12.7)
Admission to NICU[c]	1 (2.2)	0 (0)	NA	NA
Congenital anomaly according to the ICD-10 classification	5 (11.1)	3 (8.1)	0.72	1.37 (0.35, 5.36)
Nasal polyp (Q30.8)	1 (2.2)	0 (0)	NA	NA
Undescended testicle (Q53.10)	1 (2.2)	0 (0)	NA	NA
Congenital anteversion hip (Q65.8) + forced position foot (Q66.89)	1 (2.2)	0 (0)	NA	NA
Craniosynostosis (Q75.0)	0 (0)	1 (2.7)	NA	NA
Strawberry naevus (Q82.5)	0 (0)	1 (2.7)	NA	NA
Accessory nipple (Q83.3)	0 (0)	1 (2.7)	NA	NA
Congenital hypertrichosis (Q84.2)	1 (2.2)	0 (0)	NA	NA
Down syndrome (Q90) + congenital duodenum stenosis (Q41.0)	1 (2.2)	0 (0)	NA	NA

Data are presented as *n* (%).

[a] At the time of follow-up all children were alive.

[b] PP: per protocol analysis. As shown in FIGURE 1 one woman assigned to the modified NC-FET group eventually received AC-FET in the ANTARCTICA RCT. Therefore the analysis is presented as per protocol.

[c] There was one admission to the neonatal intensive care unit (NICU) after neonatal resuscitation following placental abruption during premature delivery.

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The ANTARTICA trial study group consists of: Myrthe L Bandell, Albert Schweitzer Hospital, Department of Obstetrics and Gynecology, Zwijndrecht, the Netherlands; Egbert A Brinkhuis, Meander Medical Center, Department of Obstetrics and Gynecology, Amersfoort, the Netherlands; Kathrin Fleisher, University Medical Center Nijmegen, Department of Obstetrics and Gynecology, Nijmegen, the Netherlands; Annemieke Hoek, Department of Obstetrics and Gynecology, University Medical Center Groningen, Groningen, the Netherlands; Diederik A Hoozemans, Medisch Spectrum Twente, Department of Obstetrics and Gynecology, Enschede, the Netherlands; Eugenie M Kaaijk, Onze Lieve Vrouwe Gasthuis, Department of Obstetrics and Gynecology, Amsterdam, the Netherlands; Carolina AM Koks, Máxima Medical Center, Department of Obstetrics and Gynecology, Veldhoven, the Netherlands; Esther B Baart, Department of Obstetrics and Gynecology, Erasmus Medical Center Rotterdam, the Netherlands; Paul JQ van der Linden, Deventer Hospital, Department of Obstetrics and Gynecology, Deventer, the Netherlands; Petra A Manger, Diakonessenhuis, Department of Obstetrics and Gynecology, Utrecht, the Netherlands; Minouche ME van Rumste, Catharina Hospital, Department of Obstetrics and Gynecology, Eindhoven, the Netherlands; Taeke Spinder, Medisch Centrum Leeuwarden, Department of Obstetrics and Gynecology, Leeuwarden, the Netherlands; Jantien Visser, Amphia Hospital, Department of Obstetrics and Gynecology, Breda, the Netherlands.

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SUPPLEMENTARY MATERIALS

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