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A 5-year multicentre randomized controlled trial comparing personalized, frozen and fresh blastocyst transfer in IVF

BIOGRAPHY

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KEYWORDS

Endometrial receptivity
Endometrial receptivity analysis (ERA)
Fresh embryo transfer (ET)
Frozen embryo transfer (FET)
Personalized embryo transfer (PET)
Window of implantation (WOI)

KEY MESSAGE

This study demonstrates the clinical potential of personalized embryo transfer guided by the endometrial receptivity analysis test at the first appointment, which should be confirmed in larger randomized controlled trials.

ABSTRACT

Research question: Does clinical performance of personalized embryo transfer (PET) guided by endometrial receptivity analysis (ERA) differ from frozen embryo transfer (FET) or fresh embryo transfer in infertile patients undergoing IVF?

Design: Multicentre, open-label randomized controlled trial; 458 patients aged 37 years or younger undergoing IVF with blastocyst transfer at first appointment were randomized to PET guided by ERA, FET or fresh embryo transfer in 16 reproductive clinics.

Results: Clinical outcomes by intention-to-treat analysis were comparable, but cumulative pregnancy rate was significantly higher in the PET (93.6%) compared with FET (79.7%) ($P = 0.0005$) and fresh embryo transfer groups (80.7%) ($P = 0.0013$). Analysis per protocol demonstrates that live birth rates at first embryo transfer were 56.2% in PET versus 42.4% in FET ($P = 0.09$), and 45.7% in fresh embryo transfer groups ($P = 0.17$). Cumulative live birth rates after 12 months were 71.2% in PET versus 55.4% in FET ($P = 0.04$), and 48.9% in fresh embryo transfer ($P = 0.003$). Pregnancy rates at the first embryo transfer in PET, FET and fresh embryo transfer arms were 72.5% versus 54.3% ($P = 0.01$) and 58.5% ($P = 0.05$), respectively. Implantation rates at first embryo transfer were 57.3% versus 43.2% ($P = 0.03$), and 38.6% ($P = 0.004$), respectively. Obstetrical outcomes, type of delivery and neonatal outcomes were similar in all groups.

Conclusions: Despite 50% of patients dropping out compared with 30% initially planned, per protocol analysis demonstrates statistically significant improvement in pregnancy, implantation and cumulative live birth rates in PET compared with FET and fresh embryo transfer arms, indicating the potential utility of PET guided by the ERA test at the first appointment.

INTRODUCTION

When reproductive success or failure relies on collaboration between two partners, the functionality and synchronization of both are necessary. One reason why IVF treatments are not successful when good-quality embryos or even euploid embryos are transferred into the endometrial cavity (*Rubio et al., 2017*) may be due to the endometrial factor. Globally, IVF remains inefficient, with current live birth rates of only 25–30% per started cycle (*Adamson et al., 2018*); for comparison, rodents or rabbits have more than 95% implantation rate. The main difference lies in the endometrial and decidual control in human implantation events, versus embryo control in non-menstruating species (*Simon and Giudice, 2017*).

The endometrial mucosa is the anatomic and functional cellular mediator for embryonic implantation. Women menstruate in an endless resetting process to synchronize the uterus for the imminent arrival of a blastocyst. Progesterone is the single most important hormone controlling the initiation and course of pregnancy inducing the acquisition of a 12–48-h transient modification of the endometrial epithelium known as the window of implantation (WOI), demonstrated clinically (*Navot et al., 1991*), epidemiologically (*Wilcox et al., 1999*), and morphologically (*Murphy et al., 2004*). This limited period can be found from LH + 6 to LH + 9 in a natural cycle or from progesterone + 4 to progesterone + 7 in a hormonal replacement therapy (HRT) cycle (*Ruiz-Alonso et al., 2013*; *2014*).

The transition from anatomical to molecular medicine has demonstrated the feasibility of an objective molecular classification of the human endometrium using transcriptomic profiling throughout the menstrual cycle (*Ponnampalam et al., 2004*; *Talbi et al., 2006*) as well as during the WOI (*Riesewijk et al., 2003*). Precision medicine is focused on targeted individualized treatment. On this basis, in 2011, we reported the transcriptomic signature of human endometrial receptivity from 238 genes with an artificial intelligence platform and algorithm able to identify the WOI of a given patient, leading to the creation of the endometrial receptivity analysis (ERA) (*Diaz-Gimeno et al., 2011*; *2013*). The clinical utility of ERA is in guiding a personalized embryo transfer (PET) by synchronizing the embryo with the WOI to the patient in a personalized manner for the initial indication of implantation failure of endometrial origin (*Ruiz-Alonso et al., 2013*; *2014*; *Mahajan et al., 2015*; *Hashimoto et al., 2017*; *Tan et al., 2018*). The goal is to improve clinical implantation by personalizing, diagnosing and synchronizing the endometrial factor.

In this multicentre, international, randomized controlled trial (RCT), infertile patients undergoing clinical evaluation for IVF were also assessed whether PET guided by the ERA test increased the probability of live birth, pregnancy and implantation rates versus frozen embryo transfer (FET) or fresh embryo transfer.

MATERIALS AND METHODS

Study design

An RCT evaluating PET guided by the ERA test versus FET or fresh embryo transfer. Participants were recruited from 16 reproductive clinics in Europe, USA and Asia, and randomized to one of the three arms indicated from 25 November 2013 to 30 April 2017. All additional embryo transfers of the remaining cryopreserved embryos in the PET and FET arms as well as new fresh embryo transfers in the embryo transfer arm carried out within 1 year after the first embryo transfer were also included in the estimation of cumulative rates. Subsequently, all participants who conceived were followed through pregnancy up to delivery; obstetric and neonatal outcomes were also reported.

Data on pregnancy outcome were prospectively collected from the clinical sites involved and entered them into a secure electronic database.

Ethical approval

Ethical approval was given by Instituto Valenciano de Infertilidad Ethics Committee (2 July 2013), Spain; Local Ethics Commission of SBALAGRM – Sofia (29 January 2014), Bulgaria; UZ Brussels Medical Ethics Committee, Belgium (26 February 2014 with code 2014/006); Instituto Conmemorativo Gorgas de Estudios de la Salud Ethics Committee Panama 26 February 2014 with code 214/CBI/ICGES/14); Istanbul Bilim Üniversitesi Klinik Arastirmalari Ethics Committee, Turkey (28 May 2014 with code 28.05.2014/20-145); Hospital das Clínicas da Faculdade de Medicina da USP Ethics Committee Brazil 3 December 2015 with code 1.349.189; Oak Clinic Group's Ethics Committee, Japan (28 February 2016). Each participating site provided a site-specific approval, and all participants provided written informed consent. Data were monitored by a clinical research associate. The protocol was first registered on 7 October 2013, and the first patient recruited on 25 November 2013 (ClinicalTrials.gov ID NCT01954758).

Participants

Infertile patients undergoing IVF were recruited at their first appointment. Women scheduled for embryo transfer at the blastocyst stage (day 5 or 6) were included. Inclusion criteria were age 37 years or younger, body mass index (BMI) of 18.5–30 and normal ovarian reserve (antral follicle count ≥8 and FSH <8 IU/ml). Ovarian stimulation protocol was decided by the enrolling physician, and the blastocyst vitrification system was the one used by the clinic. Couples who had experienced recurrent miscarriage (more than two previous biochemical pregnancies or spontaneous abortions), implantation failure (more than three failed IVF cycles with good-quality embryos transferred) or who had severe male factor infertility (fewer than 2 million spermatozoa/ml) were not eligible. Pathology affecting the endometrial cavity, including polyps and submucosal myomas, intramural myomas over 4 cm or hydrosalpinx previously operated on before embryo transfer, was not an exclusion criterion. Couples using preimplantation genetic diagnosis for aneuploidy (PGT-A) could also participate.

Post-randomization exclusion criteria were progesterone levels of over 1.5 ng/ml on the day of HCG administration in all groups, absence of blastocysts (day 5 or 6) for embryo transfer, or risk of ovarian hyperstimulation syndrome (OHSS) in the fresh embryo transfer arm. For all these cases, patients did not enter the per protocol analysis but were included in the intention-to-treat analysis.

During the first appointment in each participating site and after providing informed consent, suitable patients were randomized into either PET, FET or fresh embryo transfer groups. In the FET group, patients received transfer during a hormonal replacement therapy (HRT) cycle after embryo thawing, following the protocol and timing used in each clinic.

Ovarian stimulation, embryo culture and vitrification

In all groups, ovarian stimulation was carried out using standard protocols in each of the participant sites according to female age, basal hormone levels, basal ovarian reserve and BMI. After a normal basal ultrasound, gonadotrophins were administered from day 2–3 after menstruation. Serial transvaginal ultrasound examinations and serum oestradiol determination were started on day 5 of ovarian stimulation and repeated every 48 h to monitor the ovarian response. Ovulation triggering was carried out mainly with HCG administration or gonadotrophin releasing hormone agonist, depending on the gonadotrophin protocol used, when at least two follicles reached 18 mm. Oocyte retrieval was scheduled 36 h later.

Intracytoplasmic sperm injection (ICSI) or IVF was carried out according to the protocols of the participating sites, and fertilization was assessed 17–20 h after insemination or microinjection. Embryos were cultured using different approaches and culture media depending on the IVF laboratory. Embryo morphology and quality were evaluated according to Gardner's criteria (*Gardner et al., 2007*). Properly developed blastocysts were transferred on day 5 or 6 in the fresh embryo transfer arm, or vitrified in the PET and FET arms, using different protocols depending of the IVF laboratory. Subsequent blastocyst transfer was carried out at each participant site with different types of transfer catheters.

Personalized embryo transfer guided by endometrial receptivity analysis

In the PET group, patients underwent one or two endometrial biopsies (the timing of the second biopsy depended on the first result), and embryo transfer was carried out in an HRT cycle at the timing indicated by the ERA test.

Endometrial biopsies were collected from the uterine fundus using a Pipelle catheter from Cornier® devices (CCD Laboratories, Paris, France) or similar, under sterile conditions. After the biopsy, the endometrial tissue was transferred to a cryotube containing 1.5 ml of RNALater (QIAGEN, Barcelona, Spain), vigorously shaken for a few seconds, and kept at 4°C or in ice for at least 4 h. The samples were then shipped at room temperature for ERA test.

Endometrial preparation for frozen embryo transfer and personalized embryo transfer

The endometrium was prepared for the cycle to carry out the ERA test, and subsequent PET and FET were carried out using an HRT cycle, as previously described (*Ruiz-Alonso et al., 2013*;*2014*). Briefly, after menstruation, ovarian quiescence was confirmed by vaginal ultrasound evaluation and oestradiol administration started from the second or third day onwards. Sonographic evaluation and oestradiol and progesterone assessment were carried out between 7 and 10 days after endometrial oestradiol preparation treatment. When a trilaminar endometrium measuring 6 mm or more with an endogenous progesterone serum level close to zero was observed, progesterone was administered at dosage and route of the participant physician or clinic for a period of 5 days (progesterone + 5 or 120 h, approximately).

Fresh embryo transfer

Embryo transfer in fresh cycles was carried out 5 or 6 days after oocyte retrieval according to blastocyst timing. Luteal phase supplementation route and dosage was the one used by the participant physician or clinic.

Sample labelling, hybridization and personalized embryo transfer recommendation

Total RNA was extracted using the TRIzol method according to the manufacturer's protocol (Life Technologies, Inc.,

Carlsbad, CA, USA). About 1–2 µg of total RNA were obtained per mg of endometrial tissue. RNA quality was assessed by loading 300 ng of total RNA onto an RNA Labchip and analysing it in an A2100 Bioanalyzer according to manufacturer's protocol (Agilent Technologies, Inc., Santa Clara, CA, USA). Good-quality RNA samples, with RNA integrity number greater than 7, was a prerequisite for ERA. Sample preparation and hybridization was adapted from the Agilent technical manual (one-color). In short, first strand cDNA was transcribed from 200 ng of total RNA using T7-Oligo(dT) Promoter Primers. Samples were transcribed *in vitro,* and Cy-3 labelled, all with a Low Input Quick Amp Labeling kit (Agilent Technologies, Inc., Santa Clara, CA, USA). The labelling reaction typically yielded between 4 and 5 µg of complementary RNA with a specific activity greater than 6. Fragmented complementary RNA samples were hybridized onto the customized ERA microarray (*Diaz-Gimeno et al., 2011*), by incubation at 65°C for 17 h with constant rotation. The microarray was then washed with two 1-min steps in two different washing buffers (Agilent Technologies, Inc., Santa Clara, CA, USA). Hybridized microarrays were scanned in an Axon 4100A scanner (Molecular Devices, Sunnyvale, CA, USA), and data were extracted with the GenePix Pro 6.0 software (Molecular Devices, Sunnyvale, CA, USA). Endometrial receptivity analysis gene expression values were pre-processed and normalized, and the endometrial receptivity status was diagnosed by the ERA computational predictor (*Díaz-Gimeno et al., 2011*). The ERA test diagnoses an endometrial sample as receptive, pre-receptive or post-receptive with an associated diagnostic probability. This provides a recommendation for PET in a particular patient at P + 4, P + 4.5, P + 5, P + 5.5, P + 6, or P + 7. As described previously (*Díaz-Gimeno et al., 2011*), the customized microarray for ERA was designed after selecting 238 genes involved in human endometrial receptivity based on different gene expression profiles across the receptive, pre-receptive and post-receptive stages using raw expression data generated for a previous publication from our group. Genes showing an absolute fold-change greater than 3 and a false discovery rate less than 0.05 were selected. Three statistical approaches were used to generate the prediction: the union of the T-Rex gene list (GEPAS) (http://gepas.bioinfo.cipf.es/) and SAM gene list (http://www.stat.stanford.edu/_tibs/SAM/), intersected with the multtest gene list (http://www.bioconductor.org/). Selected genes were included in a customized gene expression microarray with a 15Kx8 format by using 4.5 earray (https://earray.chem.agilent.com/earray/). All the probes included in ERA were selected from Agilent's catalogue to avoid crosslinking phenomena. The ERA predictor was based on support vector machine prediction with a specificity and sensitivity of 0.886 and 0.998, respectively (*Diaz-Gimeno et al., 2011*).

Outcome measures

Primary outcome was live birth rate (LBR) after the first embryo transfer. Secondary outcomes were cumulative LBR (CLBR), pregnancy rate and implantation rate, both at the first embryo transfer and cumulative rates after 1-year follow-up. Percentages of biochemical pregnancy, clinical miscarriage and ectopic pregnancy from the total number of beta-HCG-positive patients as well as obstetric, delivery and neonatal outcomes were also reported.

According to the International Glossary on Infertility and Fertility Care (*Zegers-Hochschild, et al., 2017*), the following standardized definitions were considered.

Pregnancy rate

Pregnancy rate is the number of patients with positive serum level of beta-HCG (≥25 mIU/ml) per embryo transfer.

Live birth rate

The LBR is the number of deliveries that resulted in at least one live birth per embryo transfer. Live birth is defined as the complete expulsion or extraction from a woman of a product of conception after 22 weeks of gestation, which, after such separation, breathes or shows any other evidence of life, such as heart beat, umbilical cord pulsation or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached.

Implantation rate

The implantation rate is the number of gestational sacs observed by vaginal ultrasound at the fifth gestational week divided by the number of embryos transferred.

Clinical miscarriage rate

The clinical miscarriage rate is the number of spontaneous pregnancy losses in which a gestational sac or sacs was previously observed, per number of pregnancies.

Biochemical pregnancy rate

The biochemical pregnancy rate is the number of pregnancies diagnosed only by beta-HCG detection without a gestational sac visualized by vaginal ultrasound at the fifth week of pregnancy, per number of pregnancies.

Ectopic pregnancy rate

The ectopic pregnancy rate is the number of pregnancies outside the uterine cavity, diagnosed by ultrasound, surgical visualization or histopathology, per number of pregnancies. For cumulative outcomes, we considered the clinical results obtained from all the embryo transfers performed in the same arm of the study up to 12 months follow-up.

Cumulative pregnancy rate

The cumulative pregnancy rate (CPR) is the number of patients with positive serum level of beta HCG 25 mIU/ml or above divided by the total number of patients receiving embryo transfer after the same type of transfer arm into which the patient was randomized for up to 12 months' follow-up.

Cumulative live birth rate

The CLBR is the number of deliveries that resulted in at least one live birth (as previously defined), per total number of patients receiving embryo transfer following the same type of transfer arm into which the patient was randomized for up to 12 months' follow-up.

All clinical outcomes are presented as percentages. Patients with positive pregnancy tests were followed to delivery. Patients without pregnancy, clinical or biochemical miscarriages, or both, or ectopic pregnancies, were followed up to 12 months after the first embryo transfer to calculate the cumulative outcomes.

Sample size calculation and statistical analysis

Sample size calculation was based on the hypothesis of 15-percentage point absolute difference in primary and secondary endpoints (alpha = 5%; beta = 20%) in the study group (PET) versus FET and fresh embryo transfer.

This analysis was carried out using a SPSS Macro for one-sided significance level analysis, resulting in a sample size of at least 130 patients per group. Because of the multicentre and international nature of the study, a 30% drop-out rate was estimated owing to possible loss to follow-up, no blastocyst for transfer or no protocol compliance. Therefore, a plan was made to recruit a total of 546 patients, corresponding to 182 patients per group. This was a non-blinded study; all participants were assigned to each of the three arms using a simple equal-probability randomization method. A software-based application was used to allocate intervention (1:1:1) with randomization stratified by site.

Analyses on an intention-to-treat and per protocol basis were conducted for all data. Repeated measures analysis of variance was used to compare non-categorical variables among the three groups, multiple comparisons and post-hoc tests (Bonferroni and two-sided Dunnett) were applied for each one to one group comparisons. Mean differences and standard deviation or median and interquartile ranges were used when the variables were not homogeneous, as well as the mean difference with 95% CI values. Chi-squared test and two-sided Fisher's exact test were used to compare the study groups with respect to percentages. Differences were estimated as relative risks with 95% CI. We conducted a multivariable regression analysis with a binomial endpoint (live birth Yes/No) to demonstrate the homogeneity of the key baseline variables and the absence of bias towards our final endpoint. We included all baseline variables presented in TABLE 1 as follows: age, body mass index, site, FSH, anti-Müllerian hormone, ethnicity and fertility background.

$P < 0.05$ was considered to be statistically significant. SPSS 25 software (IBM, MD, USA) was used for statistical analysis.

RESULTS

Between 25 November 2013 and 30 April 2017 569 patients were assessed for eligibility (FIGURE 1). One hundred and eleven patients were excluded from randomization because they did not meet the inclusion criteria ($n = 51$), declined to participate ($n = 43$) or were double-randomized by mistake ($n = 8$), or an inclusion error was detected ($n = 9$). The remaining 458 women were randomly assigned to either PET guided by the ERA ($n = 148$), FET ($n = 154$) or fresh embryo transfer) ($n = 156$).

Loss to follow-up occurred in 24 patients, 27 did not receive blastocyst transfer (16 had no blastocyst, six spontaneous pregnancies occurred, two were cancelled owing to OHSS risk, and, in three cases, no embryo transfer data were found), and 139 did not fully comply with the protocol (seven had no PET in the PET group, six had PET in the non-PET groups, 43 had high progesterone, 21 had fresh embryo transfer on day 2, 3 or 4, 10 and seven had fresh and frozen embryo transfer in the PET group, 22 had fresh embryo transfer in the FET group, six had frozen embryo transfer in the embryo transfer group, 13 had frozen embryo transfer owing to OHSS risk in the fresh embryo transfer group, four protocol deviations for other reasons). The intention to treat (ITT) analysis was conducted in 434 patients, PET ($n = 141$), FET ($n = 148$) or fresh embryo transfer ($n = 145$). Per protocol analysis was conducted in 266 patients (PET [$n = 80$], FET [$n = 92$] or fresh embryo transfer [$n = 94$]) (FIGURE 1).

Baseline demographics and clinical characteristics were comparable among groups, both at the ITT and per protocol analysis (TABLE 1). The FSH values were similar between PET and FET groups and higher compared with fresh embryo transfer group. About 71% of the patients recruited at their first appointment had no previous IVF attempts.

Intention-to-treat analysis of cycle characteristics and embryological data were also broadly similar in all groups (TABLE 2). A statistically significant lower percentage of day-5 blastocysts, however, was transferred in the PET group compared with fresh embryo transfer (69.5% versus 82.1%, respectively; $P = 0.01$). Similar features were observed by per protocol analysis, where a significantly lower percentage of day-5 blastocysts was transferred in the PET and FET group compared with fresh embryo transfer (85.0% and 84.8% versus 96.8%, respectively; $P = 0.01$ (Supplementary Table 1), together with a significantly lower number of transferred embryos in the PET versus embryo transfer arms (1.38 versus 1.6; $P = 0.01$). No morphological differences were recorded in embryo quality in terms of inner cell mass or trophectoderm grading.

Clinical outcomes at the first embryo transfer and cumulative rates up to 12 months by ITT analysis are shown in TABLE 3. In the PET group, CPR was significantly higher (132/141 [93.6%]) compared with FET (118/148 [79.7%]) and fresh embryo transfer (117/145 [80.7%]) groups (PET versus FET RR 1.17, 95% CI 1.07 to 1.29, $P = 0.0005$; PET versus fresh embryo transfer RR 1.16, 95% CI 1.06 to 1.27, $P = 0.0013$). Significantly more miscarriages took place in the PET than the fresh embryo transfer group at the first attempt (20.5% versus 5.9% $P = 0.006$) and cumulative miscarriage rate (18.2% versus 4.3%, $P = 0.0006$) (TABLE 3).

Per protocol analysis is presented in TABLE 4. At first embryo transfer, LBR was 45 out of 80 (56.2%) in the PET group, 39 out of 92 (42.4%) in the FET group, and 43 out of 94 (45.7%) in the fresh embryo transfer group (PET versus FET RR 1.33, 95% CI 0.98 to 1.80, $P = 0.09$; PET versus fresh embryo transfer RR 1.23, 95% CI 0.92 to 1.65, $P = 0.17$). The number of patients needed to treat (NNT) by ERA (PET) to achieve the estimated LBR versus the control groups (FET and fresh embryo transfer) was calculated according to NNT= 100/Io-Ie (where Io is the LBR in the control group and Ie is the LBR in the study group). NNT of PET versus FET was 7.2 patients (Io = 42.4; Ie = 56.2), and NNT of PET versus fresh embryo transfer was 9.5 patients (Io = 45.7; Ie = 56.2); thus, these were the numbers of patients needed to treat by ERA (PET) to achieve one additional delivery with at least one live birth versus control groups.

The CLBR was significantly higher in the PET group (71.2%) compared with FET (55.4%) and fresh embryo transfer (48.9%) groups (PET versus FET, RR 1.28, 95% CI 1.02 to 1.62, $P = 0.04$; PET versus fresh embryo transfer RR 1.46, 95% CI 1.13 to 1.87, $P = 0.003$).

Pregnancy rate at the first embryo transfer was statistically significantly higher in the PET group versus FET. Pregnancy rate was 72.5% in the PET group, 54.3% in the FET group and 58.5% in the fresh embryo transfer group (PET versus FET, RR 1.33, 95% CI 1.06 to 1.68, $P = 0.01$; PET versus fresh embryo

TABLE 1 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE PATIENTS AT BASELINE

	PET (*n* = 148)	FET (*n* = 154)	Fresh embryo transfer (*n* = 156)
Age, years	33 ± 3.1	32.8 ± 3.4	32.7 ± 3.3
Body mass index[a]	22.8 ± 2.9	22.9 ± 2.9	22.6 ± 2.8
Ethnicity, *n* %			
White	122 (82.4)	127 (82.5)	129 (82.7)
Asian	12 (8.1)	12 (7.8)	11 (7.1)
Latin American	13 (8.8)	11 (7.1)	13 (8.3)
African	0 (0.0)	4 (2.6)	1 (0.6)
Other or unknow	1 (0.6)	0 (0.0)	2 (1.2)
Current smoker	15 (10.1)	12 (7.8)	15 (9.6)
Fertility history			
Duration of infertility, years	3.1 ± 1.9	3.2 ± 2.1	2.9 ± 2.2
Previous IVF failed, *n* (%)			
0	109 (73.6)	104 (67.5)	112 (71.8)
1	20 (13.5)	23 (14.9)	22 (14.1)
2	10 (6.7)	10 (6.5)	12 (7.7)
3	6 (4.0)	11 (7.1)	6 (3.8)
Unknown	3 (2.0)	6 (3.9)	4 (2.6)
Previous deliveries, *n* (%)			
1	11 (7.4)	16 (10.3)	17 (10.9)
≥2	3 (2.0)	4 (2.6)	3 (1.9)
Spontaneous clinical miscarriages, *n* (%)			
1	23 (15.5)	26 (16.9)	24 (15.4)
≥2	6 (4.0)	3 (1.9)	0 (0.0)
Elective abortions, *n* (%)	3 (2.0)	9 (5.8)	8 (5.1)
Previous curettages (1 or 2), *n* (%)	12 (8.1)	11 (7.1)	10 (6.4)
Ectopic pregnancies, *n* (%)	8 (5.4)	3 (1.9)	4 (2.6)
IVF indication, *n* %			
Male factor	65 (43.9)	78 (50.6)	50 (32.1)
Tubal factor	20 (13.5)	31 (20.1)	33 (21.1)
PCOS	27 (18.2)	20 (12.9)	14 (9.0)
Ovarian disorders	4 (2.7)	5 (3.2)	7 (4.5)
Endometriosis	21 (14.2)	9 (5.8)	13 (8.3)
Unexplained	33 (22.3)	33 (21.4)	47 (30.1)
Other or unknown[b]	2 (1.3)	4 (2.6)	10 (6.4)
Laboratory tests			
FSH (mU/ml)	5.9 ± 1.9[c]	6.6 ± 2.1	6.9 ± 2.0[d]
AMH (ng/nl)	4.4 ± 3.6	3.7 ± 2.7	3.5 ± 2.9

Data are expressed as mean ± SD unless indicated otherwise.

[a] Body-mass index is the weight in kilograms divided by the square of the height in metres.

[b] Other indications included same sex couples, single women, cervical factor, anatomical factor and male genetic disease.

[c,d]Statistically significant differences between groups at <0.05 level. No significant differences were found in any of the baseline characteristics except for FSH between PET and fresh embryo transfer ($P = 0.003$).

Analysis of variance, Bonferroni and two-sided Dunnett tests were used to compare numerical variables in the three groups. Chi-Squared and Fisher's exact tests were used to compare categorical variables.

AMH, anti-Müllerian hormone; FET, frozen embryo transfer; PCOS, polycystic ovary syndrome.; PET, personalized embryo transfer.

transfer, RR 1.24, CI 1 to 1.54, $P = 0.057$). Statistically significant differences were also found in implantation rate in PET (57.3%) versus FET (43.2%) and fresh embryo transfer (38.6%) groups (PET versus FET, RR 1.33, 95% CI 1.03 to 1.70, $P = 0.03$; PET versus fresh embryo transfer, RR 1.48, 95% CI 1.14 to 1.92, $P = 0.004$). Cumulative pregnancy rate in the PET group (95%) was significantly higher than FET (70.6%) and fresh embryo transfer (62.8%) groups (PET

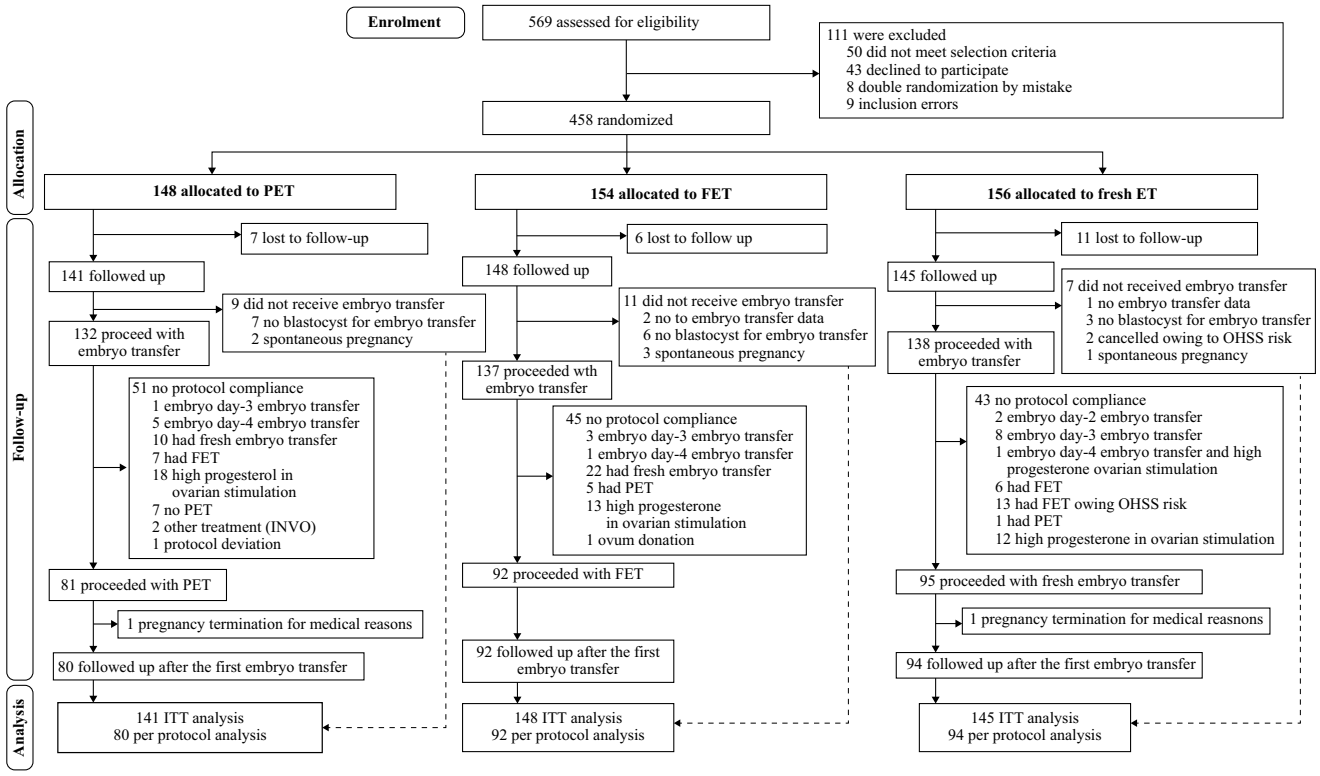

FIGURE 1 CONSORT flow diagram: endometrial receptivity analysis randomized controlled trial. FET, frozen embryo transfer; INVO, intravaginal culture of oocytes; ITT, intention-to-treat; OHSS, ovarian hyperstimulation syndrome; PET, personalized embryo transfer.

versus FET, RR 1.34, 95% CI 1.17 to 1.55, $P < 0.0001$; PET versus fresh embryo transfer, RR 1.51, 95% CI 1.28 to 1.78, $P < 0.0001$) (TABLE 4). For cumulative clinical outcomes (after the first attempt), most of the pregnancies and live births occurred in the second and third embryo transfer (Supplementary Table 2). No differences were found in miscarriages, biochemical or ectopic pregnancies among the three groups investigated (TABLE 4). Obstetrical outcomes, type of delivery and neonatal outcomes were not statistically significant different in all groups analysed (TABLE 5). The binomial multivariable regression analysis did not show any statistical relation between LBR and the rest of variables included in the study (data not shown).

Subgroup analysis of clinical outcome was investigated according to the number of previous failed IVF cycles. The LBR and pregnancy rate in the PET group were consistently 10 or more percentage points higher in all categories regardless of the previous failed cycles but did not reach significance owing to the final sample size (Supplementary Table 3). Clinical results were also analysed according to time from endometrial biopsy carried out for the ERA test to transfer for a period of 9 months. Pregnancy rate and LBR were not statistically different throughout this period, ruling out any beneficial or detrimental effect of the injury carried out at endometrial biopsy (Supplementary Table 4).

The ERA biopsy was obtained after 118.3 ± 4.6 h of progesterone administration (range 108 to 128 h). Results showed that 50 patients (62.50%) had the WOI at P + 5, whereas 30 out of 80 (37.5%) had a displaced WOI (early receptive [$n = 5$], late receptive [$n = 18$] and pre-receptive [$n = 7$]). In the PET arm, patients underwent embryo transfer according to ERA results; primary and secondary clinical outcomes were similar irrespective of whether PET was carried out in non-displaced (56% LBR [$n = 50$]) or displaced (56.7% LBR [$n = 30$]) WOI (Supplementary Table 5).

Cost-effectiveness per baby born in Europe and the USA was also calculated in each arm of the study, using as reference the mean cost of procedures in these two geographical locations. This assessment considered the cost of the different procedures involved in each arm of the study. In all arms, the cost of monitoring, retrieval, transfer, drugs and IVF laboratory was included. In FET and PET, the cost of embryo vitrification and HRT monitoring with laboratory work was added. Finally, in the PET arm, the cost of the ERA test plus the additional HRT was incorporated. The cost per baby in the PET arm considering the technology used in the present study was lower than the FET arm (17% in Europe and 15% in USA, approximate) and higher compared with fresh embryo transfer (25% in Europe and 16% in USA, approximate) (TABLE 6).

DISCUSSION

This international, multicentre RCT was designed to test the effect of the endometrial factor assessed by the ERA test and clinically translated as PET. The first inclusion criterion was patients aged 37 years or younger to avoid advanced maternal age as a possible cause of embryo aneuploidies that could bias the results because of the embryonic factor; the same rationale applies for antral follicle count 8 or over and FSH less than 8 IU/ml to avoid the inclusion of poor responders. In these overall good-prognosis patients, clinical outcomes at the first embryo transfer and cumulative

TABLE 2 CYCLE CHARACTERISTICS AND EMBRYOLOGICAL DATA: INTENTION-TO-TREAT ANALYSIS

	PET (*n* = 141)	FET (*n* = 148)	Fresh embryo transfer (*n* = 145)
Antral follicle count	14.8 ± 6.3	14.9 ± 6.6	13.1 ± 5.9
Antagonist protocol, *n* (%)	124 (87.9)	120 (81.1)	122 (84.1)
Agonist protocol, *n* (%)	10 (7.1)	13 (8.8)	12 (8.3)
Unknown, *n* (%)	7 (5.0)	15 (10.1)	11 (7.6)
Total dose of FSH administered, IU	1696.9 ± 687.8	1540.2 ± 635.2	1666.1 ± 669.8
Total dose of HMG administered, IU	1167.03 ± 936	1202.3 ± 987	1165.1 ± 1042.5
Progesterone level at the day of ovulation triggering	1.02 ± 0.7	0.93 ± 0.6	0.92 ± 0.8
Ovulation triggering, *n* (%)			
HCG	62 (44.0)[c]	57 (38.5)[c]	110 (75.9)[d]
Agonist	62 (44.0)[c]	67 (45.3)[c]	15 (10.3)[d]
Double triggering	7 (5.0)	7 (4.7)	7 (4.8)
Oocytes retrieved	12.4 ± 7.6	11.6 ± 6.0	10.5 ± 6.6
Fertilization technique, *n* (%)			
ICSI	106 (75.2)	114 (77.0)	111 (76.6)
IVF	5 (3.5)	6 (4.1)	9 (6.2)
IVF/ICSI	21 (14.9)	13 (8.8)	21 (14.5)
INVO	2 (1.4)	0 (0.0)	0 (0.0)
Unknown	7 (5.0)	15 (10.1)	4 (2.8)
Fertilization rate, *n* (%)	1244/1633 (76.2)	1197/1531 (78.2)	1067/1379 (77.4)
Embryo stage, *n* (%)			
Cleavage	1/181 (0.6)	0 (0.0)[c]	7/211 (3.3)[d]
Morula	2/181 (1.1)	1/208 (0.5)	1/211 (0.5)
Early blastocyst	12/181 (6.6)	11/208 (5.3)	5/211 (2.4)
Cavitated blastocyst	40/181 (22.1)	47/208 (22.6)	48/211 (22.7)
Expanded blastocyst	93/181 (51.4)	100/208 (48.1)	109/211 (51.7)
Hatching blastocyst	33/181 (18.2)	49/208 (23.6)	41/211 (19.4)
Blastocyst development rate, *n* (%)	648/1248 (51.9)	636/1239 (51.3)	561/1093 (51.3)
Day of embryo development at transfer, *n* (%)[a]			
2	0 (0.0)	0 (0.0)	2 (1.4)
3	10 (7.1)	4 (2.7)	10 (6.9)
4	7 (5.0)[c]	4 (2.7)	0 (0.0)[d]
5	98 (69.5)[c]	112 (75.7)	119 (82.1)[d]
6	16 (11.3)	17 (11.5)[c]	6 (4.1)[d]
Unknown, *n* (%)	10 (7.1)	11 (7.4)	8 (5.5)
Embryo quality (known/total embryos), *n*	149/201	183/220	183/225
Inner cell mass, *n* (%)			
A grade	48/149 (32.2)	70/183 (38.3)	56/183 (30.6)
B grade	84/149 (56.4)	92/183 (50.3)	110/183 (60.1)
C grade	17/149 (11.4)	21/183 (11.5)	17/183 (9.3)
Trophectoderm, *n* (%)			
A grade	36/149 (24.2)	56/183 (30.6)	46/183 (25.1)
B grade	85/149 (57)	95/183 (51.9)	96/183 (52.5)
C grade	28/149 (18.8)	32/183 (17.5)	40/183 (21.9)
PGT-A cases	6 (4.3)	4 (2.7)	3 (2.1)
Number of embryos per transfer	1.52 ± 0.5	1.61 ± 0.5	1.63 ± 0.5

(continued on next page)

TABLE 2 – (continued)

	PET (*n* = 141)	FET (*n* = 148)	Fresh embryo transfer (*n* = 145)
Warmed HRT embryo transfer data			
Days of oestradiol, *n*	15.5 ± 3.8[c]	16.6 ± 3.8[c]	NA
Endogenous progesterone levels[b]	0.2 (0.03–1.4)	0.29 (0.05–11.03)	NA
Exogeneous progesterone administration, h	120 ± 14.4	117.8 ± 9.7	NA
Exogeneous progesterone administration, h, range	65.2–163.4 (98.2)	66.4–151.2 (84.8)	NA
Time between ovarian stimulation and embryo transfer, months	3.2 ± 2.4[c]	2.1 ± 1.4[d]	NA

Data are expressed as mean ± SD unless otherwise specified.

[a] Total number of indicated cases may differ from the excluded number of cases indicated in **FIGURE 1** because some patients had more than one cause for exclusion.

[b] Progesterone value (ng/ml) before its administration is represented as median (interquartile range).

[c,d]Statistically significant differences between groups at the <0.05 level. Significant differences were found among groups in ovulation triggering ($P = 0$), embryo stage (cleavage embryo, $P = 0.005$), day of embryo development ($P = 0.006$), number of days of oestradiol ($P = 0.03$) and time between ovarian stimulation and embryo transfer (months) ($P = 0.002$). No significant differences were found in the other variables.

Analysis of variance, Bonferroni and two-sided Dunnett tests were used to compare numerical variables in the three groups, whereas Independent samples Student's t-test was used to compare quantity variables between group PET and FET regarding warmed HRT embryo transfer data. Chi-squared and Fisher's exact tests were used to compare categorical variables.

FET, frozen embryo transfer; HMG, human menopausal gonadotrophin; HRT, hormone replacement therapy; ICSI, intracytoplasmic sperm injection; INVO, intravaginal culture of oocytes; PET, personalized embryo transfer; PGT-A, preimplantation genetic diagnosis for aneuploidy.

rates up to 12 months by ITT analysis were not statistically significant in the three arms of the study, except that CPR that was significantly higher in the PET group compared with FET and fresh embryo transfer groups, and clinical miscarriages were significantly higher in PET versus fresh embryo transfer.

When the analysis was carried out per protocol, LBR significance in PET versus FET and fresh embryo transfer was not achieved at the first embryo transfer (56.2%, 42.4%, 45.7%, respectively; PET versus FET, $P = 0.09$; PET versus fresh embryo transfer, $P = 0.17$). Significantly higher CLBR after 12 months' follow-up from PET versus FET and fresh embryo transfer, however, were observed (71.2%, 55.4%, 48.9%, respectively; PET versus FET, $P = 0.04$; PET versus fresh embryo transfer; $P = 0.003$). Implantation rate at first attempt was significantly higher in PET compared with FET and fresh embryo transfer. In addition, pregnancy rate after PET was significantly higher than FET at the first attempt and significantly higher than FET and fresh embryo transfer at cumulative rate (**TABLE 4**).

The delivery of a healthy baby may take several treatment cycles; therefore, the CLBR per patient is a key outcome variable. To ensure that results are presented without bias, a statistical comparison was made of CLBR for each of the four cycles carried out in each arm of the study regardless of the follow-up time (Supplementary Table 2). The significant difference in CLBR in PET can be observed at the second embryo transfer.

In the PET arm, outcomes were similar in the non-displaced ($n = 50$; LBR 56%) and the displaced group ($n = 30$; LBR 56.7%), indicating the importance of a timely PET. More than 45,500 data and variables corresponding to the 569 patients enrolled in the 16 active sites across continents were recorded in the study Case Report Form. These data were verified according to each patient source document during the continuous on-site and remote monitoring visits. This exhaustive source data verification allowed us to detect and correct mistakes and protocol deviations as well as to resolve queries, ensuring the study data integrity and accuracy.

The study was powered to detect statistical differences for a 15-percentage point increase in the primary and secondary outcomes in the PET group guided by ERA versus FET or fresh embryo transfer. This unexpected dilution was the main reason why LBR significance in PET versus FET and fresh embryo transfer was not achieved at the first embryo transfer (56.2%, 42.4%, 45.7%, respectively; PET versus FET, $P = 0.09$; PET versus fresh embryo transfer; $P = 0.17$) (**TABLE 4**).

The significantly lower number of blastocysts transferred in the PET arm versus fresh embryo transfer (1.38 versus 1.6; $P = 0.01$), as well as the lower percentage of day-5 blastocysts (85% versus 96.8%; $P = 0.01$), reinforces the concept of the positive effect of the personalization of endometrial factor even with embryonic 'inferiority' (Supplementary Table 1).

Personalized medicine is still in its infancy in reproductive medicine, but most specialists in the field will agree that 'one size does not fit all'. In fact, clinicians and embryologists have been evaluating precision medicine through assisted reproductive technology treatment for decades (*Mol et al., 2018*), from the starting dose and dose adjustment of gonadotrophins in ovarian stimulation according to ovarian reserve, age and BMI; determination of oocyte retrieval timing based on ultrasound findings and oestradiol and progesterone levels; selection of the insemination and fertilization technique (ICSI, IVF, or both) according to sperm features and clinical background; or embryo selection based on morphological grading, chromosomal analysis of the cleavage embryos, or both, or blastocyst.

Interestingly, endometrial status at the time of embryo transfer was guided in recent decades by endometrial thickness assessed through transvaginal ultrasound together with administration or supplementation of progesterone or HCG as luteal phase support. Endometrial thickness, however, is not a diagnostic test for endometrial receptivity. Indeed, this approach has limited capacity to identify patients with low possibility to conceive after embryo transfer, except for atrophic or hyperplasic endometrium (*Kasius et al., 2014*). Therefore, the use of

TABLE 3 REPRODUCTIVE OUTCOMES AT FIRST EMBRYO TRANSFER AND CUMULATIVE OUTCOMES DURING 1-YEAR FOLLOW-UP: INTENTION-TO-TREAT ANALYSIS

	PET (*n* = 141)	FET (*n* = 148)	Fresh embryo transfer ET (*n* = 145)	PET versus FET		PET versus fresh embryo transfer	
				Relative risk (95% CI)	*P*-value	Relative risk (95% CI)	*P*-value
Transfers, *n*	132	137	138				
Pregnancy rate, *n* (%)	83 (58.9)	73 (49.3)	84 (57.9)	1.19 (0.96 to 1.48)	0.12	1.02 (0.84 to 1.24)	0.9
Implantation rate, *n* (%)	88/201 (43.8)	80/220 (36.4)	97/225 (43.1)	1.20 (0.95 to 1.52)	0.14	1.02 (0.82 to 1.26)	0.92
Live birth rate *n* (%)	57 (40.4)	51 (34.5)	64 (44.1)	1.17 (0.87 to 1.58)	0.33	0.92 (0.70 to 1.20)	0.55
Singleton	49/57 (86)	40/51 (78.4)	45/64 (70.3)	1.1 (0.92 to 1.31)	0.32	1.22 (1.01 to 1.48)	0.049
Multiple (all twins)	8/57 (14)	11/51 (21.6)	19/64 (29.7)	0.65 (0.28 to 1.49)	0.32	0.47 (0.22 to 1)	0.049
Clinical miscarriages, *n* (%)	17/83 (20.5)	11/73 (15.1)	5/84 (5.9)	1.36 (0.68 to 2.71)	0.41	3.44 (1.33 to 8.90)	0.006
Biochemical pregnancies, *n* (%)	7/83 (8.4)	9/73 (12.3)	11/84 (13.1)	0.68 (0.27 to 1.74)	0.44	0.64 (0.26 to 1.58)	0.46
Ectopic pregnancies, *n* (%)	1/83 (1.2)	1/73 (1.4)	1/84 (1.2)	0.88 (0.06 to 13.82)	1	1.01 (0.06 to 15.92)	1
Elective termination of pregnancy, *n* (%)	1/83 (1.2)	0/73 (0.0)	1/84 (1.2)				
Neonatal mortality, *n* (%)	0/83 (0.0)	1/73 (1.4)	0/84 (0.0)				
Live birth lost to follow-up, *n* (%)	0 (0.0)	0 (0.0)	2/84 (2.4)				
Patients with additional embryo transfers, *n* (%)	57 (40.4)	57(38.5)	42 (28.9)	1.05 (0.79-1.40)	0.81	1.40 (1.01 to 1.93)	0.047
Total additional cycles and transfers, *n*	150	130	110				
Cumulative transfers, *n*	282	267	248				
Pregnancies from additional embryo transfers, *N*	49	45	33				
Cumulative pregnancy rate, *n* (%)	132/141 (93.6)	118/148 (79.7)	117/145 (80.7)	1.17 (1.07 to 1.29)	0.0005	1.16 (1.06 to 1.27)	0.0013
Cumulative live birth rate, *n* (%)	88/141 (62.4)	82/148 (55.4)	85/145 (58.6)	1.13 (0.93 to 1.37)	0.23	1.06 (0.88 to 1.28)	0.55
Singleton	75/88 (85.2)	67/82 (81.7)	58/85 (68.2)	1.04 (0.91 to 1.19)	0.54	1.25 (1.06 to 1.48)	0.011
Multiple (all twins)	13/88 (14.8)	15/82 (18.3)	27/85 (31.8)	0.81 (0.41 to 1.59)	0.54	0.47 (0.26 to 0.84)	0.011
Cumulative clinical miscarriages, *n* (%)	24/132 (18.2)	17/118 (14.4)	5/117 (4.3)	1.26 (0.71 to 2.23)	0.49	4.26 (1.68 to 10.79)	0.0006
Cumulative biochemical pregnancies, *n* (%)	19/132 (14.4)	16/118 (13.6)	23/117 (19.7)	1.06 (0.57 to 1.97)	1	0.73 (0.42 to 1.27)	0.31
Cumulative ectopic pregnancies, *n* (%)	1/132 (0.8)	1/118 (0.8)	1/117 (0.9)	0.89 (0.06 to 14.14)	1	0.89 (0.06 to 14.02)	1
Transfers per patient	2.63 ± 1.14	2.28 ± 0.70	2.62 ± 0.73	0.35 (–0.4 to 0.4)	0.1	0.01 (–0.43 to 0.45)	1

Values are expressed as mean ± SD unless otherwise specified. Fisher's exact test (two-sided) was used to compare these results. Relative risk (95% CI) was calculated using the approximation of Katz. Independent-samples Student's t-test was applied when quantitative variables were compared; relative risk and 95% CI were calculated using Bonferroni and Dunnett T3 post hoc tests.

endometrial thickness as an actionable biomarker to decide whether to cancel cycles, freeze all embryos or refrain from further IVF treatment is not justified based on the current data. Luteal phase supplementation starts around the time of oocyte retrieval after HCG trigger, although a start on the day of the oocyte retrieval does not improve clinical outcomes compared with a start 6 days later (*Connell et al., 2015*). Furthermore, much effort has been dedicated to comparing clinical results using different routes, dosages or duration of progesterone administration in IVF/ICSI cycles, in an endless pharmacological dispute, resulting in no evidence favouring any of them (*Glujovsky et al., 2010*).

Local injury to the endometrium during the ERA biopsy is suggested to help increase implantation success in the subsequent month. We analysed clinical results according to time from endometrial biopsy for the ERA test to embryo transfer for a period of 9 months, concluding that pregnancy rate and LBR were statistically non-significant throughout this period (Supplementary Table 4) and ruling out any beneficial or detrimental effect of the injury occurring at the endometrial biopsy. Similar findings were reported in a previous prospective study in patients who had experienced recurrent implantation failure, in which patients were followed after biopsy for ERA to transfer for a period of 6 months; no improvement in reproductive outcomes was detected the month after endometrial biopsy (*Ruiz-Alonso et al., 2013*). The discussion about the clinical value of endometrial scratching is coming to end after an RCT involving 1364 women undergoing IVF reported that scratching did not result in a higher rate of live birth than no intervention (*Lensen et al., 2019*).

Critically, the objective diagnosis of the endometrial receptivity factor remains

TABLE 4 REPRODUCTIVE OUTCOMES AT THE FIRST EMBRYO TRANSFER AND CUMULATIVE OUTCOMES DURING 1-YEAR FOLLOW-UP: PER PROTOCOL ANALYSIS

	PET (*n* = 80)	FET (*n* = 92)	Fresh embryo transfer (*n* = 94)	PET versus FET		PET versus fresh embryo transfer	
				Relative risk (95% CI)	*P*-value	Relative risk (95% CI)	*P*-value
Pregnancy rate, *n* (%)	58 (72.5)	50 (54.3)	55 (58.5)	1.33 (1.06 to 1.68)	0.01	1.24 (1 to 1.54)	0.057
Implantation rate, *n* (%)	63/110 (57.3)	60/139 (43.2)	58/150 (38.6)	1.33 (1.03 to 1.70)	0.03	1.48 (1.14 to 1.92)	0.004
Live birth rate, *n* (%)	45 (56.2)	39 (42.4)	43 (45.7)	1.33 (0.98 to 1.80)	0.09	1.23 (0.92 to 1.65)	0.17
Singleton	40/45 (88.9)	30/39 (76.9)	33/43 (76.7)	1.16 (0.95 to 1.41)	0.16	1.16 (0.95 to 1.41)	0.16
Multiple (all twins)	5/45 (11.1)	9/39 (23.1)	10/43 (23.2)	0.48 (0.18 to 1.32)	0.16	0.48 (0.18 to 1.28)	0.16
Clinical miscarriages, *n* (%)	9/58 (15.5)	7/50 (14)	3/55 (5.4)	1.11 (0.44 to 2.76)	1	2.84 (0.81 to 9.97)	0.13
Biochemical pregnancies, *n* (%)	4/58 (6.9)	4/50 (8)	8/55 (14.5)	0.86 (0.23 to 3.27)	1	0.47 (0.15 to 1.49)	0.23
Ectopic pregnancies, *n* (%)	0 (0.0)	0 (0.0)	1 (1.8)				
Patients with surplus embryo transfers, *n* (%)	19 (23.7)	16 (17.4)	4 (4.2)				
Total surplus embryo transfers, *n*	39	18	10				
Cumulative transfers, *n*	119	110	104				
Pregnancies from surplus embryo transfers, *n*	18	15	4				
Cumulative pregnancy rate, *n* (%)	76/80 (95)	65/92 (70.6)	59/94 (62.8)	1.34 (1.17 to 1.55)	<0.0001	1.51 (1.28 to 1.78)	<0.0001
Cumulative live birth rate, *n* (%)	57 (71.2)	51 (55.4)	46 (48.9)	1.28 (1.02 to 1.62)	0.04	1.46 (1.13 to 1.87)	0.003
Singleton	51/57 (89.5)	41/51 (80.4)	34/46 (73.9)	1.11 (0.95 to 1.31)	0.28	1.21 (1 to 1.47)	0.066
Multiple (all twins)	6/57 (10.5)	10/51 (19.6)	12/46 (26.1)	0.54 (0.21 to 1.37)	0.28	0.40 (0.16 to 0.99)	0.066
Cumulative clinical miscarriages, *n* (%)	10/76 (13.2)	8/65 (12.3)	3/59 (5.1)	1.07 (0.45 to 2.55)	1	2.59 (0.75 to 8.99)	0.15
Cumulative biochemical pregnancies, *n* (%)	9/76 (11.8)	6/65 (9.2)	9/59 (15.3)	1.28 (0.48 to 3.41)	0.78	0.78 (0.33 to 1.83)	0.62
Cumulative ectopic pregnancies, *n* (%)	0 (0.0)	0 (0.0)	1/59 (1.7)				
Transfers per patient	3.05 ± 1.61	2.13 ± 0.34	3.5 ± 1.29	0.92 (–0.11 to 1.97)	0.09	–0.45 (–2.13 to 1.24)	1

Values are expressed as mean ± SD unless otherwise specified. Fisher's exact test (two-sided) was used to compare these results. Relative risk (95% CI) was calculated using the approximation of Katz. Independent samples Student's t-test was applied when quantitative variables were compared; relative risk and 95% CI were calculated using Bonferroni and Dunnett T3 post hoc tests.

FET, frozen embryo transfer; PET, personalized embryo transfer.

neglected. Endometrial receptivity analysis was the first molecular test created for this purpose to assess a personalized clinical approach for the endometrial factor, aiming to improve clinical success from the endometrial perspective in patients who had experienced recurrent implantation failure (*Ruiz-Alonso et al., 2013*; *2014*; *Hashimoto et al., 2017*). As a next step, we tested in this RCT the quantitative relevance of the personalization of the endometrial factor in patients attending the infertility clinic at their first appointment. In the work-up of patients at the initial visit, we generally include general semen analysis, AMH, FSH, prolactin and thyroid-stimulating hormone. In this RCT, we also included the ERA test to objectively diagnose patients' individual WOI and to guide PET; in this way, we could incorporate endometrial factor in the IVF process in addition to embryo quality. To avoid bias caused by variations in natural cycles, HRT was used for consistency. The ERA test, however, has been validated for natural and modified natural cycles (*Diaz-Gimeno et al., 2011*; *2013*; *Ruiz-Alonso et al., 2013*).

Embryo quality was assessed morphologically, and PGT-A was neither an inclusion nor an exclusion criterion. To address whether PGT-A might have biased study outcomes, we assessed the relevance of this factor in detail. First, the number of PGT-A cases did not significantly differ between the PET group and the other two arms. By ITT analysis, there were 13 PGT-A cases in a total of 434 randomized patients (3%): six out of 141 in the PET arm, four out of 148 in the FET arm and three out of 145 in the fresh embryo transfer arm (4.3%, 2.7% and 2.1%, respectively; $P = 0.6$). By per protocol analysis, nine PGT-A cases in a total of 266 patients were included (3.4%): five out of 80 in the PET arm, three out of 92 in the FET arm and one out of 94 in the fresh embryo transfer arm (6.3%, 3.3% and 1.1%, respectively, $P = 1.17$). Therefore, in this RCT, the percentage of PGT-A embryo transfers was negligible (3.4%), and evenly distributed among the three arms of the study, i.e., not statistically significantly different.

Of note, per protocol, we registered a 5.4% miscarriage rate after fresh embryo transfer compared with 15.2% in PET and 14% in FET. According to the International Committee for Monitoring Assisted Reproductive Technologies world report (*Dyer et al., 2016*), pregnancy loss in fresh embryo

TABLE 5 OBSTETRICAL, DELIVERY AND NEONATAL OUTCOMES: PER PROTOCOL ANALYSIS

	PET (*n* = 80)		FET (*n* = 92)		Fresh embryo transfer (*n* = 94)		PET versus FET		PET vs fresh embryo transfer	
	Outcomes, *n*		Outcomes, *n*		Outcomes, *n*		Relative risk (95% CI)	*P*-value	Relative risk (95% CI)	*P*-value
Ovarian hyperstimulation	80	0 (0.0)	92	0 (0.0)	94	1 (1.1)				
Obstetrical outcomes	45		39		43					
Gestational diabetes		2 (4.4)		1 (2.6)		1 (2.3)				
High blood pressure		1 (2.2)		0 (0.0)		0 (0.0)				
Placenta previa		1 (2.2)		1 (2.6)		0 (0.0)				
Retrocorial haematoma		0 (0.0)		1 (2.6)		1 (2.3)				
Abruption		1 (2.2)		0 (0.0)		0 (0.0)				
Vasa previa		1 (2.2)		0 (0.0)		0 (0.0)				
Still birth		1 (2.2)		1 (2.6)		0 (0.0)				
Type of delivery	40		35		43					
Caesarean section		10 (25.0)		14 (40.0)		15 (34.9)	0.62 (0.32 to 1.22)	0.22	0.72 (0.36-1.41)	0.35
Vaginal		30 (75.0)		21 (60.0)		28 (65.1)	1.25 (0.90 to 1.73)	0.22	1.15 (0.87-1.53)	0.35
Neonatal outcomes[a]	40		35		43					
Neonatal mortality		0 (0.0)		1 (2.9)		0 (0.0)				
Gestational age, weeks	38	38.03 ± 3.1	34	38.03 ± 2.9	42	38.33 ± 1.6	−0.003 (−1.49 to 1.48)	1	-0.3 (-1.72-1.10)	1
Preterm birth <37 weeks	38	5 (13.2)	34	6 (17.6)	42	4 (9.5)	0.75 (0.25 to 2.22)	0.75	1.38 (0.40-4.77)	0.73
Birth weight, g	23	3170.6 ± 646.9	30	2868.5 ± 629.134		2912.6 ± 573.6	302.15 (−112.7 to 717)	0.23	258 (-146.1-662.2)	0.36
Birth weight in singletons	17	3484.4 ± 321.6	14	3362.5 ± 402	22	3210.68 ± 375.6	121.91 (−205.8 to 449.6)	1	273.7 (-19.5-566.9)	0.07
Birth weight in twins	6	2281.7 ± 476.716		2436.2 ± 444.9	12	2366.2 ± 463.2	−154.58 (−707.9 to 398.7)	1	-84.58 (-662.5-493.3)	1
Birth weight <2500 g	23	4 (17.4)	30	10 (33.3)	34	6 (17.6)	0.52 (0.19 to 1.45)	0.22	0.99 (0.31-3.11)	1
Birth height, cm	17	49.9 ± 2.7	27	48.3 ± 2.6	30	48.9 ± 2.3	1.69 (−0.23 to 3.61)	0.1	1.09 (-0.79-2.97)	0.48
APGAR score										
1 min	28	8.7 ± 1.5	21	9 ± 0.8	18	9.22 ± 0.7	−0.32 (−1.12 to 0.48)	0.98	-0.54 (-1.38-0.29)	0.34
5 min	24	9.5 ± 0.9	16	9.9 ± 0.2	12	9.7 ± 0.4	−0.48 (−1.03 to 0.07)	0.11	-0.29 (-0.89-0.31)	0.71
Congenital anomalies		0 (0.0)		0 (0.0)		0 (0.0)				

Values are given as mean ± SD unless otherwise specified. Fisher's exact test (two-sided) was used to compare qualitative variables; relative risk (95% CI) was calculated using the approximation of Katz. Independent samples Student's t-test was used to compare quantitative variables; relative risk and 95% CI were calculated using Bonferroni and Dunnett T3 post hoc tests.

[a] Percentage is ratio of total deliveries.

transfer cycles occurred at a rate of 21.8% (2008), 21.1% (2009) and 20.2% (2010). Corresponding early pregnancy loss rates for FET were 28.9% (2008), 25.4% (2009) and 25.2% (2010). On the basis of these data, the unexpected 5.4% miscarriage rate after fresh embryo transfer was below the rate reported in published research worldwide. We are unsure how to explain it, but this finding would be expected to bias the results toward favouring fresh embryo transfer versus the other two arms of the study. Nevertheless, biochemical pregnancies occurred in eight out of 55 (14.5%) in the fresh embryo transfer group higher than PET (6.9%) and FET (8%), balancing the total pregnancy losses of around 20% in all arms (22.1% in PET, 22% in FET and 19.9% in fresh embryo transfer). This observation suggests that biochemical pregnancies might be a reflection of the endometrial status.

In the PET arm, 30 patients (37.5%) had a diagnosis of WOI displacement, and PET was carried out with a different

TABLE 6 COST-EFFECTIVENESS ESTIMATION PER LIVE-BORN BABY AT THE FIRST ATTEMPT

	PET (*n* = 80)		FET (*n* = 92)		Fresh embryo transfer (*n* = 94)	
Deliveries with at least one live birth at the first attempt, *n*	**45**		**39**		**43**	
	EU, €	**USA, $**	**EU, €**	**USA, $**	**EU, €**	**USA, $**
IVF laboratory cost	5190[a]	11,825[a]	5190[a]	11,825[a]	5590[b]	12,325[b]
Drug cost	1700[c]	5500[c]	1600 [d]	4700[d]	1580 [e]	4500[e]
Vitrification cost	1100[f]	1,375[f]	1100[f]	1375[f]	–	–
Additional cost in PET and FET	2050 [g]	3500[g]	2050 [g]	3500[g]	–	–
Cost of ERA	710	795	–	–	–	–
Mock cycle	250[h]	1000[h]	–	–	–	–
Total cost per embryo transfer	11,000	23,995	9940	21,400	7170	16,825
Estimated cost of a delivery with at least one live birth at the first attempt	19,555	42,658	23,448	50,482	15,674	36,780

Data are expressed as mean.

The detailed cost presented in TABLE 1 corresponded to the cost of the different steps of the treatment in the patients included in the study (Euros and Dollars).

[a] Monitoring (bloodwork and ultrasounds), retrieval, anaesthesia, intracytoplasmic sperm injection, extended culture and male partner seminogram.

[b] Monitoring (bloodwork and ultrasounds), retrieval, anaesthesia, intracytoplasmic sperm injection, extended culture, male partner seminogram and transfer.

[c] Gonadotrophins, HCG, progesterone x2, oestradiol x2.

[d] Gonadotrophins, HCG, progesterone x1, oestradiol x1.

[e] Gonadotrophins, HCG, progesterone x1.

[f] Cryopreservation and storage.

[g] Monitoring (bloodwork and ultrasounds), warming and transfer.

[h] Monitoring (bloodwork and ultrasounds) and biopsy.ERA, endometrial receptivity analysis; FET, frozen embryo transfer; PET, personalized embryo transfer.

timing than for standard WOI; 17 out 30 patients (56.7%) achieved a live birth in their first attempt. Similar clinical outcome was obtained for patients in whom the WOI was present at the expected timing (Supplementary Table 5). Considering that there should be a similar percentage of patients with displaced WOI in the other study arms that were blind to the endometrial factor assessment, this might explain the differential results obtained.

Finally, using the clinical results obtained in this RCT, we conducted a cost-effectiveness study per live birth with reference to economic figures for Europe and the USA, where the IVF costs are quite different. This assessment demonstrated that the estimated cost per live birth in the PET arm is approximately 17% less compared with the most expensive FET and 25% more than the less costly FET (15% and 17%, respectively in the USA) (TABLE 6).

The main limitation of our study is the unexpected 50% patient drop-out rate versus 30% initially planned. This situation has rendered the study underpowered to detect statistical significance by ITT analysis in the PET arm versus FET and fresh embryo transfer, except for higher CLBR. This sample size affected the per protocol analysis; although the analysis detected a 13.8 and 10.5-percentage points increase in LBR in the PET group versus FET and fresh embryo transfer at the first embryo transfer, the difference was not significant because the study was powered to detect statistical differences for a 15-percentage point increase in the primary and secondary outcomes. Second, in the historical setting in which this RCT was designed, ERA was carried out using microarray technology with early algorithms, and in some cases two endometrial biopsies were needed for diagnosis. At the time of publication, ERA is being done by next-generation sequencing combined with refined algorithms informed by the analysis and clinical follow-up of more than 50,000 endometrial samples worldwide; additionally, only one endometrial biopsy is needed. Because of the indicated constraints of the study, we are conducting a new ERA 2.0 RCT, in which the current sequencing technology and refined algorithms will be included together with proper power of the study.

In conclusion, to the best of our knowledge, this is the first RCT aiming to provide proof-of-principle evidence for the potential of using a personalized diagnosis of the endometrial factor in the work-up of the infertile couple at the first appointment. While the ITT analysis shows no beneficial effect of the ERA test except for a statistically significant CPR compared with FET and fresh embryo transfer, the per protocol analysis demonstrates a significant improvement in pregnancy rates at the first and cumulative rates up to 12 months, and implantation rates at the first attempt, indicating the potential of the ERA test to diagnose the endometrial factor in the work-up of the infertile couple. These findings need to be confirmed in a larger randomized clinical trial.

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SUPPLEMENTARY MATERIALS

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