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Follicular-phase endometrial scratching: a truncated randomized controlled trial

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STUDY QUESTION: Does intentional endometrial injury (scratching) during the follicular phase of ovarian stimulation (OS) increase the clinical pregnancy rate (CPR) in ART?

SUMMARY ANSWER: CPR did not vary between the endometrial injury and the control group, but the trial was underpowered due to early termination because of a higher clinical miscarriage rate observed in the endometrial injury arm after a prespecified interim analysis.

WHAT IS KNOWN ALREADY: Intentional endometrial injury has been put forward as an inexpensive clinical tool capable of enhancing endometrial receptivity. However, despite its widespread use, the benefit of endometrial scratching remains controversial, with several recent randomized controlled trials (RCTs) being unable to confirm its added value. So far, most research has focused on endometrial scratching during the luteal phase of the cycle preceding the one with embryo transfer (ET), while only a few studies investigated in-cycle injury during the follicular phase of OS. Also, the persistence of a scratch effect in subsequent treatment cycles remains unclear and possible harms have been insufficiently studied.

STUDY DESIGN, SIZE, DURATION: This RCT was performed in a tertiary hospital setting between 3 April 2014 and 8 October 2017. A total of 200 women (100 per study arm) undergoing IVF/ICSI in a GnRH antagonist suppressed cycle followed by fresh ET were included.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Participants were randomized with a 1:1 allocation ratio to either undergo a pipelle endometrial biopsy between Days 6 and 8 of OS or to be in the control group.
The primary outcome was CPR. Secondary outcomes included biochemical pregnancy rate, live birth rate (LBR), early pregnancy loss (biochemical pregnancy losses and clinical miscarriages), excessive procedure pain/bleeding and cumulative reproductive outcomes within 6 months of the study cycle.

MAIN RESULTS AND THE ROLE OF CHANCE: The RCT was stopped prematurely by the trial team after the second prespecified interim analysis raised safety concerns, namely a higher clinical miscarriage rate in the intervention group. The intention-to-treat CPR was similar between the biopsy and the control arm (respectively, 44 versus 40%, $P = 0.61$, risk difference = 3.6 with 95% confidence interval = −10.1;17.3), as was the LBR (respectively, 32 versus 36%, $P = 0.52$). The incidence of a biochemical pregnancy loss was comparable between both groups (10% in the intervention group versus 15% in the control, $P = 0.49$), but clinical miscarriages occurred significantly more frequent in the biopsy group (25% versus 8%, $P = 0.032$). In the intervention group, 3% of the patients experienced excessive procedure pain and 5% bleeding. The cumulative LBR taking into account all conceptions (spontaneous or following ART) within 6 months of randomization was not significantly different between the biopsy and the control group (54% versus 60%, respectively, $P = 0.43$).

LIMITATIONS, REASONS FOR CAUTION: The trial was stopped prematurely due to safety concerns after the inclusion of 200 of the required 360 patients. Not reaching the predefined sample size implies that definite conclusions on the outcome parameters cannot be drawn. Furthermore, the pragmatic design of the study may have limited the detection of specific subgroups of women who may benefit from endometrial scratching.

WIDER IMPLICATIONS OF THE FINDINGS: Intentional endometrial injury during the follicular phase of OS warrants further attention in future research, as it may be harmful. These findings should be taken in consideration together with the growing evidence from other RCTs that scratching may not be beneficial.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported by 'Fonds Wetenschappelijk Onderzoek' (FWO, Flanders, Belgium, 11M9415N, 1524417N). None of the authors have a conflict of interest to declare with regard to this study.

Key words: endometrial scratching / endometrial biopsy / endometrial receptivity / embryo implantation / miscarriage / IVF/ICSI

Introduction

The first clinical trials in the field of endometrial scratching have reported a doubling of the clinical pregnancy rate (CPR) and live birth rate (LBR), in the general IVF/ICSI population (El-Toukhy *et al*., 2012; Nastri *et al*., 2012), as well as in patients presenting repeated implantation failure (RIF) (Potdar *et al*., 2012). In 2015, a Cochrane review and meta-analysis concluded that scratching is potentially beneficial in case of RIF (Nastri *et al*., 2015). However, around the same time, others reported it was impossible to perform a robust meta-analysis due to the heterogeneity among the conducted trials (Panagiotopoulou *et al*., 2015). Indeed, an enormous variation does exist in terms of the timing and the technique of the scratching procedures reported thus far (e.g. in the luteal phase prior to the transfer cycle or during the cycle itself, a single or several interventions, a pipelle biopsy or a hysteroscopy). More recent data on pipelle scratching in IVF treatments have not replicated the better reproductive outcomes detected initially and have further put into question the clinical applicability of the intervention (Yeung *et al*., 2014; Eskew *et al*., 2019; Lensen *et al*., 2019a). Furthermore, studies investigating the impact of diagnostic hysteroscopy, prior to the start of IVF or following RIF, also did not show a benefit in terms of treatment outcome, although hysteroscopy is often considered as a less invasive scratch (El-Toukhy *et al*., 2016; Smit *et al*., 2016). The topic of endometrial scratching is a clearly controversial unresolved matter to which the hereafter discussed trial adds more evidence, in parallel to several other RCTs currently ongoing or awaiting publication (Nastri *et al*., 2015; van Hoogenhuijze *et al*., 2017; Lensen *et al*., 2019b).

To the best of our knowledge, only one robust study investigated a single endometrial biopsy during the early follicular phase of ovarian stimulation (OS) followed by fresh embryo transfer (ET) (Zhou *et al*., 2008). In this trial, the implantation, CPR and ongoing pregnancy rate (OPR) were shown to be significantly higher in the intervention group. Besides the study by Zhou *et al*., few studies have evaluated the effect of scratching during OS. Gibreel *et al*. performed two endometrial biopsies of which one was in the fresh ET cycle. The authors did not detect a significant difference in LBR following the intervention; albeit, regression analysis suggested a possible benefit in RIF patients (Gibreel *et al*., 2015). When local injury was performed at the moment of oocyte retrieval, a strong negative impact on all outcome parameters (from implantation rate to OPR) has been previously reported, indicating that a too short time gap between biopsy and ET may have an adverse effect (Karimzade *et al*., 2010).

This information, coupled with the accumulating controversy around the rationale and efficacy of pre-IVF endometrial scratching (Simon and Bellver 2014; van Wely 2014; Yeung *et al*., 2014), challenged us to revisit this specific type of scratching with a new randomized controlled trial (RCT), investigating not only its potential clinical value but also its side effects/complications and the possibility of a longer-term effect, which had received little attention in prior research.

Materials and Methods

Study design

The Receptivity Enhancement by Follicular-phase Renewal after Endometrial ScratcHing (REFRESH) study was a pragmatic, single-centre, two-arm randomized controlled open-label trial, performed in a university hospital setting [Centrum voor Reproductieve Geneeskunde (CRG), Universitair Ziekenhuis Brussel (UZ Brussel)]. Patients undergoing a GnRH antagonist downregulated exogenous gonadotropin OS followed by fresh ET were included in either the control or the intervention arm. In the intervention arm, women underwent an endometrial biopsy during the follicular phase, more specifically between Days 6 and 8 of OS. In the control arm, no dummy intervention took place to avoid any (even a slight) scratching effect. The trial protocol has been previously detailed elsewhere (Santos-Ribeiro *et al*., 2017).

Ethical approval and quality assurance

The study was approved by the Ethical Committee of the UZ Brussel (on 26 February 2014, with the approval number 2014/008) and performed in accordance with the endorsed guidelines. Written informed consent was obtained from all participating patients in accordance with the Declaration of Helsinki (World Medical Association, 2013). The centre in which this clinical trial was performed is fully accredited by the Association for the Accreditation of Human Research Protection Programme (AAHRPP). The trial was prospectively registered in clinicaltrials.gov under the number NCT02061228 with the EudraCT number 2014-000442-29.

Study participants

The criteria for inclusion and exclusion are shown in Table I. During the study, patients were required to refrain from continuous use of non-steroid anti-inflammatory drugs or any other medication interfering with OS, embryology, endometrial receptivity or early pregnancy.

Patient recruitment and randomization

Women starting IVF at the CRG were presented in the daily monitoring meeting and evaluated for eligibility for the study. In a consecutive manner, patients were then contacted by telephone in the beginning of the OS to receive extensive oral information with regard to the trial. Following this telephone contact, patients interested in participation were provided with written trial information (by email) and were offered the possibility to recontact the centre if any remaining questions would arise after reading. When considering participation, they had a face-to-face counselling study visit with a dedicated study nurse between Days 6 and 8 of OS. Upon written informed consent, randomization sequence and allocation was appointed using a computer-generated randomization list with a 1:1 allocation of which concealment was ensured with sequentially numbered, opaque, sealed envelopes.

Table I Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Women aged ≥18 and <40 years	Other known reasons for impaired implantation (i.e. hydrosalpinx, fibroid distorting the endometrial cavity, Asherman's syndrome, thrombophilia or endometrial tuberculosis)
Fresh ART cycle	Oocyte donation acceptors
GnRH antagonist down-regulation	Frozen egg transfers
Signed informed consent	Embryos planned to undergo embryo biopsy
	Body mass index >35 or <18
	Women already recruited for another trial on medically assisted procreation during the same cycle
	Women who have previously enrolled in the trial
	Those unable to comprehend the investigational nature of the proposed study

ART protocols

OS was initiated on Day 2 of the menstrual cycle after it was confirmed that the patient was not pregnant and had basal hormone levels. The exogenous gonadotrophins used included either recombinant FSH (rFSH) or highly purified urinary HMG, as decided by the treating physician. Pituitary suppression with GnRH antagonist begun on Day 7 of the menstrual cycle (Day 6 of exogenous stimulation) and continued with daily injections of either cetrorelix (Cetrotide®) or ganirelix (Orgalutran®). Monitoring was done by serial vaginal ultrasound scans and hormonal analyses (E_2, P, FSH and LH), starting on Days 6–8 of OS, and then adapted according to the individual endocrine profile and follicular development. As soon as three follicles of ≥17 mm were present, final oocyte maturation and ovulation were triggered with urinary hCG (Pregnyl®) or recombinant hCG (Ovitrelle®). Cumulus-oocyte complexes were collected by transvaginal aspiration ~36 h after hCG administration and conventional IVF or ICSI was performed.

ET was performed on Day 3 or on Day 5, the latter when at least four good embryo quality embryos were available on Day 3 as described previously (Papanikolaou *et al*., 2005). Progesterone was administered vaginally (Utrogestan® or Crinone®) from the day after oocyte retrieval until the time of the hCG pregnancy test and continued until 7 weeks of pregnancy in case of positive hCG tests. In case of ongoing pregnancy, patients were contacted after the study-on period to ensure final outcome and safety.

Scratching

In the intervention arm, an endometrial biopsy was performed on Days 6 to 8 of OS with a Pipelle de Cornier® (Laboratoire CCD, France). The device was introduced into the uterus until slight resistance from the fundus was felt after which the piston was withdrawn and the device rotated through 360° as it was moved up and down for four times.

Outcome measures

The primary outcome of this study was CPR with a clinical pregnancy defined as the visualization of a gestational sac during transvaginal ultrasound (Zegers-Hochschild *et al*., 2009). Secondary outcomes included biochemical pregnancy rate (BPR), LBR, early pregnancy loss (i.e. biochemical pregnancy losses and clinical miscarriages, calculated as a percentage of the hCG positive tests and clinical pregnancies, respectively), excessive procedure pain (as a dichotomous outcome; more specifically, patients were asked whether they experienced excessive pain during the procedure), post-procedure bleeding and cumulative reproductive outcomes (number of biochemical pregnancies, clinical pregnancies, early pregnancy losses and live births) taking into account all conceptions (spontaneous or following ART) within an actively monitored 6-month follow-up period following randomization. For these cumulative reproductive outcome data, women were included who performed an elective freeze-all protocol in the study cycle and then went on with frozen ET(s). Also, when an early pregnancy loss was observed in the index cycle, these women further contributed to the cumulative reproductive outcome data if they managed to restart ART treatment within this follow-up period. Instead of working with the routine definition of cumulative outcomes evaluating one entire ART cycle, we decided to for this study analyse a specific time interval to focus on a possible longer-term effect of the scratching technique, which we hypothesized to be present for 6 months after the intervention in analogy with the assumption made by Nastri *et al*. (2012).

Sample size calculation

Most of the trials published at the start of this study had associated endometrial injury with an approximate doubling of the CPR. Depending on each trial, this meant a difference in CPR ranging from 9.9 to 54.7%. Using our centre's database, we retrospectively calculated a 32% CPR for the population with the same inclusion/exclusion criteria. Using a conservative approach, we calculated the adequate sample size needed to detect an increase of 15% in the intervention group. The sample size calculation was estimated so that the trial could have an 80% power to (with a two-side Fisher's exact test and a significance level alpha of 0.05) detect an increase of 32 to 47% in CPR, accounting for up to two prespecified safety-check interim analyses (at one-third and two-thirds of recruitment). Using a 1:1 randomization ratio, each group would require approximately 180 patients, adding up to a total of at least 360 patients required for the trial. However, the trial was terminated prematurely following the second interim analysis after the recruitment of 200 patients due to safety concerns (specifically, a potentially increased risk of miscarriage in the intervention arm).

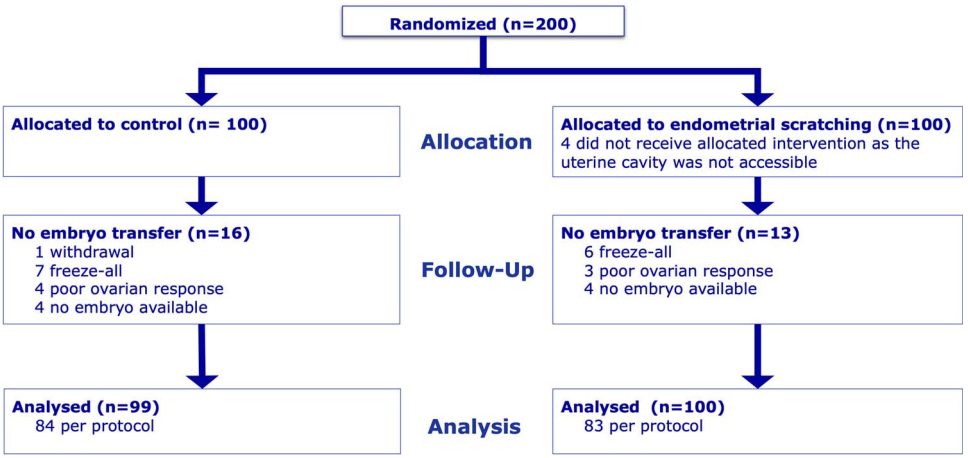

Figure 1 **Study flow chart.**

Statistical analysis

Analyses were performed in intent-to-treat (ITT) fashion and per-protocol (PP). Consequently, in the ITT analysis, all patients were included in the final analysis as long as after fulfilment of the inclusion criteria they were randomly allocated to one of the treatment groups, whereas the PP analysis included only those patients who completed the treatment originally allocated. Descriptive summary measures expressed as mean (standard deviation) were used for continuous variables and number (percentage) for categorical variables, in order to provide a summary estimate of patient demographics and baseline characteristics, in line with the CONSORT statement (www.consort-statement.org). Dichotomous outcomes were compared using the χ^2 test and continuous outcomes with either the *t*-test or Mann–Whitney test depending on the normality of their distribution. All tests were performed two-sided with Stata Software® version 13.1 (StataCorp) and a *P* value considered significant below 0.05.

Results

Study course

The trial was stopped prematurely due to safety concerns after an analysis in 200 of the required 360 patients, with 100 randomized to each arm. One patient in the control arm withdrew consent and stopped treatment for personal reasons. Consequently, the ITT analysis included 199 women (99 allocated controls versus 100 allocated interventions). In the intervention arm, four patients did not receive endometrial scratching as it was impossible to reach the uterine cavity with the pipelle without dilating the cervix. Furthermore, in both arms, 28 patients did not perform fresh ET: 13 because a freeze-all approach was applied (they were at risk for ovarian hyperstimulation or had a premature progesterone rise >1.5 ng/ml) and 15 because there was no embryo available for transfer (due to poor ovarian response or poor embryo quality). This led to a PP analysis in 167 patients (84 controls versus 83 interventions). Detailed information per study arm can be found in the study flowchart (Fig. 1).

Patient and cycle characteristics

The patient and cycle characteristics are summarized in Tables II and III. The study groups showed no statistically significant differences on the baseline characteristics. However, following randomization, the last endometrial thickness measured before ovulation triggering was statistically significantly thinner following endometrial scratching (respectively, 9.5 ± 1.9 versus 10.6 ± 2.5, $P < 0.001$).

Procedure-related complications

In 4 out of 100 women (4%) allocated to the intervention arm, it was impossible to perform the endometrial scratch as the cavity was not accessible following the standard procedure. Three of these patients reported excessive procedure-related pain. The 97 other women did not experience excessive pain or severe discomfort during the intervention, which leads to a procedure-related excessive pain incidence of 3%. Five patients (5%) reported bleeding in the time interval between the scratch and the oocyte retrieval.

Reproductive outcomes

All reproductive outcomes for the study cycle of both study arms are shown in Table IV. The primary outcome parameter, CPR, did not significantly differ between the study groups and was 40% for the control arm versus 44% for the intervention arm ($P = 0.61$, risk difference (RD) = 3.6 with 95% confidence interval (CI) = −10.1;17.3) according to the ITT analysis. BPR and LBR were also comparable for both arms, being 47 versus 49% ($P = 0.83$, RD = 1.5 with 95% CI = −12.4;15.4) and 36 versus 32% ($P = 0.52$, RD = −4.4 with 95% CI = −17.5;8.8), respectively. However, when focusing on the clinical miscarriages, significantly fewer losses were observed in the control group (8 versus 25% in the intervention group, $P = 0.032$, RD = 17.5 with 95% CI = 2.3;32.7, when calculated per clinical pregnancy and 3 versus 11%, $P = 0.028$, RD = 8.0 with 95% CI = 1.0;15.0, when calculated per randomized patient). The incidence of biochemical pregnancy losses was equal in both arms (15 versus 10%, respectively, $P = 0.49$, RD = −4.7 with 95% CI = −17.9, 8.6). The outcomes according to the PP analysis were comparable to the ITT analysis (also shown in

Table II Baseline (pre-randomization) patient and cycle characteristics of the control versus the endometrial scratching (intervention) group. Data are n (%) unless stated otherwise.

	Control	Intervention
	n = 99	*n* = 100
Maternal age (years, mean ± SD)	33.2 ± 3.7	33.1 ± 3.6
BMI (mean ± SD)	23.8 ± 3.8	22.9 ± 3.5
Indication for IVF/ICSI		
Male	36 (36.4)	37 (37.0)
Tubal	14 (14.1)	15 (15.0)
Ovulatory	17 (17.2)	17 (17.0)
Endometriosis	7 (7.1)	4 (4.0)
Idiopathic	30 (30.3)	33 (33.0)
Others	10 (10.1)	5 (5.0)
Basal FSH (mean ± SD)	7.5 ± 2.9	7.6 ± 2.2
Previous ovarian stimulation cycles (mean ± SD)	0.3 ± 0.6	0.4 ± 0.6
Ovarian stimulation		
Medication type		
rFSH	52 (52.5)	56 (56.0)
HMG	36 (36.4)	38 (38.0)
Combination	11 (11.1)	6 (6.0)
Medication dose		
Daily (IU, mean ± SD)	197.1 ± 53.1	185.8 ± 49.2
Total (IU, mean ± SD)	1848.6 ± 607.9	1736.0 ± 587.1
Duration (days, mean ± SD)	10.3 ± 1.8	10.3 1.5

Table IV). The PP analysis did not take into account patients with no fresh ET which were labelled as non-pregnant in the ITT analysis.

The cumulative reproductive outcomes taking into account all conceptions (spontaneous or following ART) within 6 months following the study cycle were analyzed to evaluate a possible longer-term effect of the scratching technique. A detailed overview of these outcomes is shown in Supplementary Table I accompanied by Supplementary Fig. 1. No significant differences were detected between the study groups for the cumulative LBR [60% in the control versus 54% in the intervention arm ($P = 0.43$, RD = −5.6 with 95% CI = −19.3;8.1)]. One spontaneous pregnancy was captured in this 6-month follow-up period, which was in a patient randomized to the control group, while all other ones were following ART (i.e. fresh and/or frozen ET).

Discussion

The REFRESH trial was prematurely interrupted due to safety concerns. Analysis of the outcome of the first 200 included women did not show a beneficial effect on the CPR, nor on the LBR, of scratching during the early follicular phase of OS followed by fresh ET, but final conclusions cannot be drawn as the predefined sample size was not reached. Nevertheless, these numbers are in contrast with a previous report in which a doubling of the OPR was described for the interventional group, although the performed procedure was similar (Zhou *et al.*, 2008). The discrepancy may be explained by the fact that in the previous study only patients with an 'abnormal' endometrium were included, defined as an endometrium with 'strong or inhomogeneous' appearance on ultrasound. Also, the study being truly randomized is a subject of discussion (Nastri *et al.*, 2015).

The current study is the first one to report a higher incidence of clinical miscarriages in the context of in-cycle scratching, which led to its premature halt. According to the sample size calculation, each study arm required at least 180 women in order to be able to perform two prespecified interim analyses. The rationale behind our decision to oversample for these extra data looks was 2-fold: first, the study team was concerned with the quality of the preceding data regarding endometrial scratching's potential benefit and, second, despite preceding data showing safety when done in the follicular phase, the result of the trial from Karimzade *et al.* (2010) on the day of oocyte retrieval also was a potential safety concern. The first prespecified look was done at 120 patients and revealed non-significant changes in both clinical pregnancy (27 vs 25 cases, $P = 0.854$) and miscarriage rates (1 vs 7 cases, $P = 0.061$), albeit with a borderline P value in the latter. Owing to unabating potential safety concerns related with risk of miscarriage, the second interim analysis was slightly anticipated to be performed following the recruitment of 200 patients. In the intervention group, 10 pregnancies resulted in a miscarriage, compared to only 3 in the control group (0.048 using the χ^2 test, $P = 0.049$ with the Fisher's exact test). With these results, it could be argued that the decision to stop the trial prematurely may be debatable given the borderline statistical significance, especially since even a single clinical miscarriage more in the control group or less in the intervention group would have rendered these P value statistically non-significant (respectively, $P = 0.096$ and $P = 0.074$). However, it is of utmost importance to stress that, in a research setting, early signs of risk to patient should always be taken seriously, since ignoring them may result in severely unethical consequences, as history has shown time and time again (Pocock, 1993; DeMets *et al.*, 1999). Moreover, while miscarriage is a relatively frequent event in ART, regulatory authorities overwhelmingly will agree that it should be considered a serious adverse event (SAE) during an RCT. Hence, when acknowledging together that (i) our preliminary negative primary outcome results were in line with other concurrent trials being published (Yeung *et al.*, 2014; Eskew *et al.*, 2019; Frantz *et al.*, 2019; Lensen *et al.*, 2019a) and (ii) there was a potential increased risk of the SAE clinical miscarriage, the lack of equipoise to continue our trial on endometrial scratching in the follicular phase became too evident to even attempt to disregard the risk using statistical considerations such as that of borderline significance.

A potential explanation for the higher clinical miscarriage rate in the scratching group may be that early follicular-phase scratching does not give the endometrium enough time to recover when immediately followed by fresh ET. In fact, in a rabbit model, it has been demonstrated histologically that complete endometrial repair following local injury can take up to 2 weeks (Li *et al.*, 2011). On the other hand, there is evidence that an endometrial biopsy would only be harmful when performed immediately prior to ET (Abate *et al.*, 1987; Ubaldi *et al.*, 1997). In this regard and in order to further elucidate the timing issue, we investigated whether an association existed with the time interval

Table III Post-randomization cycle characteristics of the control versus the intervention group. Data are mean ± SD or *n* (%)

	Control	Intervention	*P* value
	n = 99	*n* = 100	
Allocation to control/endometrial scratch			
Duration of gonadotropin treatment at the moment of endometrial scratch (days)	6.7 ± 0.6	6.9 ± 0.5	0.082
Time interval between endometrial scratch and ovulation triggering (days)	2.6 ± 1.9	2.5 ± 1.5	0.67
Time interval between endometrial scratch and embryo transfer (days)	8.5 ± 2.1	8.6 ± 1.8	0.93
Endometrial thickness			
At the moment of allocation to control/scratch (mm)	9.6 ± 2.7	9.4 ± 2.0	0.56
At last US measurement prior to ovulation trigger (mm)	10.6 ± 2.5	9.5 ± 1.9	<0.001
Lab procedure			
Number of COCs	8.6 ± 5.9	8.8 ± 5.6	0.84
IVF-ICSI-mixed-no OPU (%)	8.1-75.8-12.1-4.0	11.0-80.0-6.0-3.0	0.43
Number of fertilized oocytes	5.4 ± 4.0	5.6 ± 4.0	0.70
Number of embryos for cryopreservation	1.8 ± 2.1	1.8 ± 2.2	0.91
Stage of embryo transfer			
Number of embryos transferred	1.0 ± 0.5	1.1 ± 0.6	0.11
Embryo transfer in the study cycle?			
No: no embryo of good quality available for ET	8 (8.1)	7 (7.0)	0.22
No: freeze-all performed	7 (7.1)	6 (6.0)	
YES: fresh cleavage stage ET	48 (48.5)	36 (36.0)	
YES: fresh blastocyst stage ET	36 (36.4)	51 (51.0)	

US: ultrasound; COCs: cumulus oophorus complexes; ET: embryo transfer; OPU: oocyte pick-up.

between the day of scratching and the day of fresh ET (hypothesizing that a too short one could be harmful); logistic regression analysis however did not confirm such association (adjusted odds ratio for clinical miscarriage = 0.898, 95% CI = 0.631–1.278, *P* = 0.6). Interestingly, the incidence of having a biochemical pregnancy loss did not differ significantly between the groups. However, concerning this, it has indeed been suggested that the mechanisms causing early pregnancy loss might differ between biochemical pregnancy losses and clinical miscarriages (Salker *et al*., 2010; Vaiarelli *et al*., 2018).

Another finding of this RCT worth mentioning is the statistically significantly thinner endometrium following the scratch (Table III). Although endometrial thickness measured on Days 6 to 8 of OS was similar for both groups (9.6 ± 2.7 mm for the control versus 9.4 ± 2.0 mm for the intervention group), it was about 1 mm thinner for the study group on the last ultrasound scan performed prior to ovulation triggering (10.6 ± 2.5 versus 9.5 ± 1.9 mm, respectively); albeit, the endometrial thickness in both groups remained well above what is considered as 'normal' in case of fresh ET (Kasius *et al*., 2014; Wu *et al*., 2014; Costa-Ribeiro *et al*., 2018). It remains to be further investigated whether this finding is a result of chance alone or whether it suggests that the invasiveness of the scratching procedure, when performed as described above, may impair endometrial functionality.

Of note, 28 out of 199 patients (14%) did not proceed to fresh ET. In this regard, and given that elective frozen ET is gaining more ground (Wei *et al*., 2019), we implemented a protocol modification allowing us to monitor a 6-month follow-up period after study randomization to investigate whether there was an impact in subsequent treatment cycles. Although the presence of an immunological tissue memory has been conveyed to suggest an effect lasting for several months (McIntire *et al*., 2008; Gnainsky *et al*., 2010; Gnainsky *et al*., 2015), no significant difference was detected between the two groups in terms of cumulative reproductive outcomes within 6 months of the study cycle (Supplementary Table I and Supplementary Fig. I).

Table IV Outcome parameters of the control versus the endometrial scratching (intervention) group according to intention-to-treat and per protocol analysis. Data are *n* (%).

	Intention-to-treat			Per protocol		
	Control N = 99	Intervention N = 100	*P* value	Control N = 84	Intervention N = 83	*P* value
Biochemical pregnancy rate	47 (47)	49 (49)	0.83	47 (56)	48 (58)	0.81
Biochemical pregnancy losses	7 (15)	5 (10)	0.49	7 (15)	5 (10)	0.51
Clinical pregnancy rate	**40 (40)***	**44 (44)**	**0.61**	**40 (48)**	**43 (52)**	**0.59**
Extra-uterine pregnancies	0 (0)	1 (1.0)**	0.32	0 (0)	1 (1)**	0.31
Clinical miscarriages	**3 (8)**	**11 (25)**	**0.032**	**3 (8)**	**10 (23)**	**0.048**
Live birth rate	36 (36)	32 (32)	0.52	36 (43)	32 (39)	0.57

*Of these 40 clinical pregnancies, 3 ended up being a clinical miscarriage, 1 was a late foetal loss at 23 weeks of a monochorionic monoamniotic twin pregnancy and 36 resulted in a live birth.
**The extra-uterine pregnancy was taken into account as a clinical pregnancy, not as a miscarriage.

We acknowledge that the above-mentioned results are severely limited by the fact that the trial was stopped prior to the predetermined sample size and furthermore by the pragmatic design of the study which included a rather unselected population. Therefore, firm conclusions cannot be drawn and especially not for specific subgroups of patients. The mean number of previously performed OS cycles for patients included in the study was 0.3 (±0.6) for the control group and 0.4 (±0.6) for the intervention group, indicating that this sample set is not representative for RIF. However, even after previous failed IVF, upcoming data do not show a benefit of the procedure (Tk *et al.*, 2017; SCRaTCH trial preliminary data, NTR 5342, O-023 ESHRE 2019). A new collaborative individual patient data meta-analysis is ongoing of which the REFRESH trial forms part (PROSPERO 2017 CRD420170791 20). These results may further clarify the clinical impact of the procedure on a larger scale and for specific subpopulations.

In conclusion, the REFRESH trial detected a higher clinical miscarriage rate following early follicular phase endometrial scratching during OS followed by fresh ET in an unselected IVF/ICSI population. This observation led to a premature stop of the RCT, leaving the primary research question concerning the impact on CPR unanswered, however, warranting caution for the use of this technique in clinical practice.

Supplementary data

Supplementary data are available at Human Reproduction online.

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Authors' roles

S.M. and S.S.R. were the lead investigators of the REFRESH trial and are responsible for the concept of the study, the recruitment of the patients and the interpretation of the results. S.M. and S.S.R. wrote the manuscript. S.S.R. performed the statistical analysis. S.M., S.S.R. and A.R. performed the scratching procedures. A.R., P.D., H.V.D.V. and H.T. contributed to the interpretation and editing of the manuscript. D.S., C.B. and S.S.R. are responsible for the concept and supervised the study. All authors revised and approved the final version of the manuscript.

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Conflict of interest

None of the authors have a conflict of interest to declare with regard to this study.

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