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PROTOCOL

Endometrial receptivity enhancement through induced injury and repair during ovarian stimulation: the Receptivity Enhancement by Follicular-phase Renewal after Endometrial ScratcHing (REFRESH) trial protocol

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STUDY QUESTION: Does intentional endometrial injury (i.e. endometrial scratching) during ART enhance pregnancy rates?

SUMMARY ANSWER: We propose a randomized controlled clinical trial in women performing ART in which the intervention group will undergo an additional endometrial biopsy during exogenous ovarian stimulation.

WHAT IS KNOWN ALREADY: Although endometrial receptivity has been extensively studied, the mechanisms behind the implantation of an embryo remain largely a mystery. Intentional endometrial injury has been put forward by many researchers as an inexpensive clinical tool capable of enhancing endometrial receptivity. However, despite its widespread use, the benefit of endometrial scratching is still a contentious and unresolved issue.

STUDY DESIGN, SIZE, DURATION: Pragmatic two-arm randomized, single-centre, controlled open-label trial in women undergoing exogenous gonadotropin ovarian stimulation for ART followed by a fresh embryo transfer in a gonadotropin-releasing hormone antagonist suppressed cycle. The trial will include 360 women in total with a 1:1 allocation ratio and an expected total duration of up to 45 months.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Subjects in the intervention group will undergo an endometrial biopsy during the follicular phase, on the sixth to eighth day of exogenous stimulation. Furthermore, nested within this clinical trial, we will also evaluate whether the transcriptomic signatures of the material collected during the biopsy may accurately distinguish women who become pregnant from those who do not. These endometrial transcriptomic signatures will be assessed both immediately after the biopsy and following *in-vitro* decidualization.

MAIN RESULTS AND THE ROLE OF CHANCE: Our primary objective is to assess the effect of endometrial injury during exogenous gonadotropin ovarian stimulation on clinical pregnancy rates after ART. Secondary efficacy and safety outcomes include: live-birth delivery after 24 weeks, the endometrial transcriptomic profile among women in the intervention group, short-term safety (e.g. procedure intolerance due to pain, post-procedure bleeding) and long-term safety (e.g. cancelled transfers, miscarriage) outcomes.

LIMITATIONS, REASONS FOR CAUTION: Owing to its pragmatic design, this study may have limited power to determine one or more of our secondary outcomes and whether there are specific subgroups of women who may benefit significantly from performing endometrial scratching and endometrial transcriptomic profiling.

WIDER IMPLICATIONS OF THE FINDINGS: Despite the weak biological plausibility, heterogeneity in the existing randomized controlled trials and lack of evaluation of any potential risks associated with endometrial scratching, this procedure is still widely applied in current clinical practice. This clinical trial aims to pragmatically assess the potential benefits and harms of the generalized use of this strategy.

STUDY FUNDING/COMPETING INTEREST(S): this study has received a grant from the Research Foundation—Flanders (FWO, 1524417N). This organization has no further role in the study, namely with regards to protocol development, study conduction and evaluation of results.

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Key words: endometrial scratching / follicular-phase endometrial injury / endometrial receptivity / ovarian stimulation / assisted reproduction

Introduction and Rationale

To obtain a live-birth following ART, an intricate series of steps have to successfully occur (Huang *et al.*, 2011). Amongst these is implantation, when the blastocyst is embedded in the endometrial stroma. This process takes place during a period of the luteal phase known as the window on implantation (Granot *et al.*, 2012). Although endometrial receptivity (ER) has been extensively studied, the mechanisms involved remain largely a mystery.

During the luteal phase, progesterone (P) produced by the corpus luteum stimulates a pool of endometrial cells to differentiate into decidual cells and allow implantation. Various cytokines, adhesion molecules and growth/transcriptions factors regulate this process and an imbalance in one or more of these modulators may affect ER (Granot *et al.*, 2012). Previous studies have shown that there is a disruption of the natural endocrine function during ART caused by exogenous ovarian simulation, hindering endometrial decidualization, function and receptivity (Fatemi and Popovic-Todorovic, 2013). Specifically, the supra-physiologic sexual steroid concentrations present during ovarian stimulation seem to cause not only an abnormal proliferation and advancement in endometrial development but also the endocrine luteal phase defect present in ART (Fatemi and Popovic-Todorovic, 2013; Koot *et al.*, 2016).

The fate of the endometrium is decided according to the success of embryo implantation. In the absence of implantation, the corpus luteum will lack the positive embryonic feedback and will progressively senesce, reducing P production. This hormone inhibits the pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway, which, in the absence of P, will initiate endometrial breakdown. Hence, a series of inflammatory mechanisms are responsible for the cyclic endometrial remodelling that occurs until the ideal conditions for implantation are met.

The similarity between the 'physiologic' menstrual cycle and the mechanisms involved in the repair of endometrial injury raises the question whether artificially induced injury may have any effect on implantation. The first experiment in this field was performed in 1907, where Loeb (1907) concluded that mechanical injury caused to the endometrium of guinea pigs during the progestational phase caused rapid decidualization. These results were later confirmed in the mouse mode (Humphrey, 1969; Finn and Martin, 1972; Zhang *et al.*, 2015). Conversely, anti-inflammatory drugs seem to influence decidualization in rabbits (Hoos and Hoffman, 1983). While the exact mechanisms involved in this ER enhancement are yet to be identified, these experiments lead to the conclusion that inflammation seems to play a major role.

In humans undergoing IVF, most systematic reviews published thus far have concluded that endometrial injury is associated with a doubling of clinical pregnancy rates (CPR) and live birth rates (LBR) in both the 'general IVF' population (El-Toukhy *et al.*, 2012) and patients with a history of implantation failure (Nastri *et al.*, 2012; Potdar *et al.*, 2012). The overwhelming apparent strength of this initial evidence on 'endometrial scratching' led to its widespread use (Lensen *et al.*, 2016) and extension to other treatment modalities, namely IUI (El-Khayat *et al.*, 2015) and frozen embryo transfers (Dunne and Taylor, 2014), both with negative results. However, opposing views pointing out the weak biological plausibility, the heterogeneity in the existing randomized controlled trials (RCT), the potential for selection bias in the systematic reviews and the lack of adequate assessment of any potential risks associated with the procedure have also been put forward (Simon and Bellver, 2014; van Wely, 2014). These criticisms were later strengthened by more recent RCTs that failed to show any pragmatic benefit of endometrial scratching (Yeung *et al.*, 2014; Gibreel *et al.*, 2015). However, the topic of endometrial scratching remains a contentious and unresolved matter with multiple research groups attempting to evaluate its benefits in ongoing RCTs (Nastri *et al.*, 2015; Lensen *et al.*, 2016; van Hoogenhuijze *et al.*, 2017).

The knowledge that ovarian stimulation hinders ER has led to multiple efforts to adequately assess ER prior to embryo transfer. These research groups stem from many scientific fields, including immunology, histology, endocrinology, proteonomics and genomics (Fatemi and Popovic-Todorovic, 2013). Amongst these, customized microarrays that analyse the transcriptomic signature of freshly biopsied

secretory endometria have recently been developed (Diaz-Gimeno *et al.*, 2011). By analysing the transcriptomic expression profile of the endometrium, these microarrays can accurately discriminate between receptive and non-receptive uteri. Although this innovative approach has an enormous potential, its use as a decision-making tool during ART has been hampered thus far by an important limitation: a biopsy during the secretory phase induces endometrial injury which, although temporary, effectively precludes the transfer of an embryo during that window of implantation.

Objectives

Primary efficacy outcome

The primary objective of this study is to assess the effect of endometrial injury during exogenous gonadotropin ovarian stimulation on CPR during an antagonist suppressed IVF cycle. Clinical pregnancy (CP) was defined in accordance with the recommendations of the International Committee for Monitoring Assisted Reproductive Technology (ICMART), specifically as the visualization of a gestational sac during transvaginal ultrasound (Zegers-Hochschild *et al.*, 2009).

Secondary efficacy and safety outcomes

The secondary efficacy endpoint is live-birth delivery after 24 weeks. Furthermore, we intend to evaluate the following secondary safety outcomes:

- Short-term (e.g. procedure intolerance due to pain, post-procedure bleeding) and long-term (e.g. cancelled transfers, miscarriage) complications associated with endometrial injury.
- Compare the pathological/immunohistochemical and transcriptomic profile among women who eventually become and do not become pregnant following endometrial injury.

Other efficacy and safety outcomes

Furthermore, we intend to assess:

- The endocrine profile present during ovarian stimulation (namely, the circulating levels of estradiol (E_2) and P).
- The occurrence of adverse events (AE) and serious adverse events (SAE) until the first pregnancy test, in accordance to United States Food and Drug Administration Centre for Devices and Rediological Health (2016).

Material and Methods

Study design

We propose a pragmatic two-arm randomized, single-centre, controlled open-label trial. In summary, women undergoing exogenous gonadotropin ovarian stimulation for ART in an antagonist downregulated cycle will be included in either the control or intervention groups (Fig. 1). Subjects in the intervention group will additionally undergo an endometrial biopsy on the sixth to eighth day of exogenous stimulation.

Study population and eligibility criteria

Women undergoing ovarian stimulation for ART in an GnRH antagonist suppressed cycle in the Centrum voor Reproductieve Geneeskunde (CRG), Universitair Ziekenhuis Brussel (UZ Brussel) will be screened and invited to participate in the clinical trial. The criteria for inclusion and exclusion are listed in Table I.

Patient recruitment and randomization

All patients starting IVF are presented in the monitoring meeting held every week day. Patients eligible for recruitment will be screened and then contacted by telephone during the first 4 days of stimulation to be provided with oral and written patient information on the trial. Patients who are interested in participating will be booked a counselling study visit between Days 6 and 8 of ovarian stimulation. Those who consent to the trial following this counselling visit will be requested to sign a consent form. Following patient consent, all participants will be randomized using a computer-generated randomization list developed by a trusted partner from the study nurse department of our centre. Each entry of the list is individually sealed in a sequentially numbered an opaque envelope and allocated in that order to patients. Participating physicians will not have access to the randomization list.

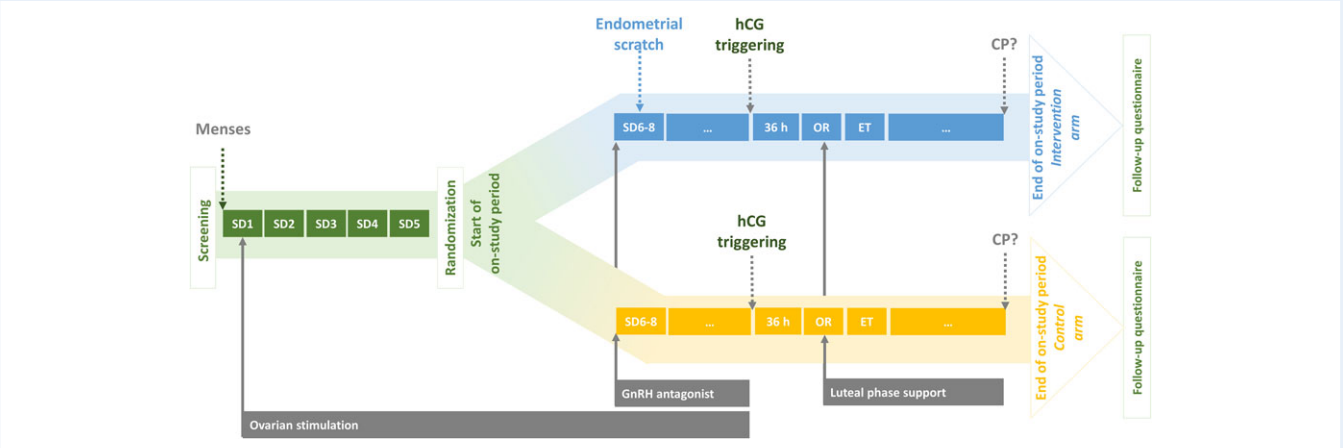

Figure 1 Study design and flowchart. SD1-8, stimulation Days 1–8; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; OR, oocyte retrieval; ET, embryo transfer; CP, clinical pregnancy.

Table I Study inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Women aged ≥18 and <40 years • Fresh ART cycle • GnRH antagonist down-regulation • Signed informed consent	• Other known reasons for impaired implantation (i.e. hydrosalpinx, fibroid distorting the endometrial cavity, Asherman's syndrome, thrombophilia or endometrial tuberculosis) • Oocyte donation acceptors • Frozen egg transfers • 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 enroled in the trial • Those unable to comprehend the investigational nature of the proposed study

Study restrictions

During the study, patients will be required to refrain from continuous use of non-steroids anti-inflammatory drugs or any other type of medication that may interfere with ovarian stimulation, embryology or early pregnancy.

Patient withdrawal, protocol violations and cycle cancellation

Patients may withdraw from the study at any time. Eligible patients who gave adequate consent to the study and later withdrew following randomization will not be replaced.

Whenever a patient does not follow the planned protocol, this will be considered as a protocol violation. This includes, for example, cancelled embryo transfers due to complications related to ovarian hyperstimulation, patient intolerance to the intervention or failure to comply with the study restrictions. The nature of the protocol violation will be recorded in the electronic case report form (eCRF) and will be accounted for during the per protocol (PP) analysis.

ART cycle cancellation is defined as any interruption of the ART process that occurs before fresh embryo transfer. Cycle cancellation will occur (i) on patient request, (ii) whenever inadequate follicular development occurs and (iii) if no embryo is available for transfer.

Financial incentives

Under the current Belgian Healthcare System, eligible subjects for this study are entitled to the reimbursement of up to six ART treatment cycles. Beyond this provision, already available to all eligible Belgian citizens, no other financial incentive will be provided to participating subjects.

Treatment common to both study arms

Owing to the pragmatic design of the study, ovarian stimulation, ultrasound and hormonal monitoring, ovulation induction, oocyte retrieval, embryology procedure, IVF and luteal support will be performed according to in the standard protocols of our centre (Fig. 1). Specifically, all women included will undergo exogenous ovarian stimulation using GnRH antagonist suppression with daily injections of either ganirelix (Orgalutran®) or cetrorelix (Cetrotide®). Treating physicians will decide on which exogenous gonadotropins should be used according to the patient's profile and preference and can include either recombinant FSH or highly purified urinary HMG. Ovarian stimulation will commence after it is confirmed that the patient is not pregnant and has basal levels of E2, P, FSH and LH. The stimulation will be monitored simultaneously by pelvic ultrasound and hormonal analyses (E2, P, FSH and LH), starting on Days 6–8 of stimulation and then every 1–3 days, according to the individual endocrine profile and follicular development.

Final oocyte maturation will be triggered with either highly purified urinary hCG (5000 UI or 10 000 UI, according to the physicians' preference and female patient weight; Pregnyl®) or 250 UI of recombinant hCG (Ovitrelle®) as soon as at least three follicles of larger than 17 mm are observed. Oocyte retrieval will be performed 36 h after hCG administration under either local anaesthesia with analgesic premedication or general anesthesia, according to patient preference.

Conventional IVF or intracytoplasmic sperm injection (ICSI) will be performed, using the specimen of sperm made available by the male progenitor on the day of oocyte retrieval.

According to embryo quality, an intrauterine embryo transfer will be performed on either the third or fifth day of development under ultrasound guidance whenever possible. Following embryo transfer, luteal support will be provided with vaginally administered P (Utrogestan®, Crinone®).

In all instances following the confirmation of an ongoing CP, women will be sent a questionnaire ~1 year after the on-study period to assess the occurrence of the secondary efficacy and safety outcomes.

Differences between the control and intervention arms

Women in the intervention group will undergo an endometrial biopsy on the sixth to eighth day of ovarian stimulation using a Pipelle de Cornier® (CCD International, Paris, France). This class I individually and sterile-packaged medical device complies with Directive 93/42/EEC and is routinely used in our centre for endometrial sampling. It is comprised of a flexible disposable polypropylene suction cannula with an outer diameter of 3.1 mm and a 2.4 mm diameter opening on the distal end. An inner plunger creates a vacuum essential for the blind endometrial biopsy.

After the introduction of the Pipelle into the uterine cavity, it will be rotated 360° and moved up and down four times after withdrawing the piston. The procedure in usually painless and otherwise inoffensive, requiring no pre- or post-medication. Slight uterine bleeding that subsides spontaneously is rare but can be seen after endometrial biopsy.

Endometrial samples collection, analysis and storage

During the conception of this trial, the possibility of performing ER analysis on the samples collected was considered to be an interesting nested study. For this reason, participating subjects will additionally be asked to specifically consent whether they authorize their biopsy material be assessed further or not. The endometrium collected during this RCT will be subdivided in four parts: (i) one for pathological/immunohistochemical evaluation (for eventual quality control, if needed), and (ii) three parts which will be snap-frozen either immediately after the biopsy (two fragments) or after stromal cell culture (1 fragment). These samples will be stored in the UZ Brussel for up to 5 years. We will evaluate the

transcriptomic signatures of the material collected, searching for variations between the women who eventually become pregnant and non-pregnant after ART treatment. These endometrial transcriptomic signatures will be assessed both immediately after the biopsy and following *in-vitro* decidualization [IVD, a technique frequently used during basic laboratorial research for ER (Teklenburg *et al.*, 2010; Weimar *et al.*, 2012) which simulates *in vitro* the development of the endometrium until the theoretical day of embryo transfer]. We hypothesize that at least one of these transcriptomic signatures will vary significantly between pregnant and non-pregnant women.

The analysis of the endometrial biopsies will be divided into two sequential steps. During the first step, we will determine and compare the endometrial transcriptomic signatures with and without IVD, while in the second step we will attempt to validate the predictive accuracy of these tools to ART outcome. Studies using transcriptomic ER profiles, thus far, have directly analysed RNA expression of the biopsy samples (Diaz-Gimeno *et al.*, 2011; Koot *et al.*, 2016). This is the approach we propose to use for the untreated late-proliferative phase samples [the specific timing during which abnormal cellular proliferation seems to be associated with failed implantation (Koot *et al.*, 2016)]. Although such samples are easier to obtain and quicker to analyse, the fact that they contain multiple cell types (e.g. epithelial, stromal, immune) may bias the expression profile, an issue potentially resolved by the culture and IVD of stromal cells alone. In other words, our objective is to compare the predictive value of transcriptomic ER analysis in two distinct settings: a pragmatic low-labour intensive approach, and a 'purer', but more labour-intensive scenario.

During the first step of this sample analysis, we will evaluate the transcriptomic signatures of the material collected before and after IVD using Next Generation RNA Sequencing (RNA-seq). This technology analyses coding RNA, non-coding RNA, specific alleles (i.e. imprinting) and splicing variants. The treatment outcome (pregnant versus non-pregnant) will be considered as a surrogate marker of ER. We will compare the different gene expression profiles of the entire human genome between receptive and the non-receptive endometria. While the late-proliferative phase biopsies will be analysed by RNA-seq without further manipulation besides cryopreservation and thawing, the post-IVD samples will be further pretreated as discussed below.

For the culture of stromal cells, the endometrial biopsy will be minced and digested (for 1 h with collagenase and DNase) to obtain a single cell suspension. The suspension will be centrifuged and the cell pellet used for incubation in a culture flask overnight. The following day, the medium will be changed and adherent endometrial stromal cells allowed to grow further until confluent. The culture will then be cryopreserved and stored for decidualization experiments. IVD will be performed after thawing the confluent culture. We will decidualize the cells with cyclic AMP and medroxyprogesterone following the protocols extensively tested at Warwick University, mimicking a luteal phase out of a proliferative biopsy. Although this technique has rarely been applied in a clinical setting, it is frequently used during basic laboratorial research to assess ER. At different time points (Days 4, 6 and 8), the cultured cells and respective supernatants will be harvested. The supernatants will be assayed for 45 cytokines, chemokine and growth factors using a multiplex suspension bead immunoassay. Meanwhile, the stomal cells will undergo quantitative real-time PCR (qRT-PCR) for implantation factors (PRL and IGF-BP1). These directed expression analyses of well-known factors related to implantation will be performed to determine the optimal time-point to perform the RNA-seq (the one revealing the highest differential expression between the pregnant and the non-pregnant groups).

Analysis of the RNA endometrial biopsies

RNA sequencing will be performed by the BRussels Interuniversity Genomics High Throughput core (BrightCore). A total RNA library of molecules with known strand origin will be prepared using the Illumina Truseq® stranded mRNA sample preparation kit. This library will be used for cluster generation on the Illumina cBOT® machine and, subsequently, paired-end sequencing (2 × 125 bp) will be performed on an Illumina HiSeq 1500®. A minimal amount of 50 million reads is expected for each sample. After obtaining the raw reads, a demultiplexing and adaptor/quality trimming step will be done. Read quality will be evaluated using the readily available online tool FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). In a next step, the reads will be mapped to the Human Genome (hg19). Alignment (exon–exon junction reads) will be performed using the open-source tool STAR (Dobin *et al.*, 2013), with the quality of this alignment being evaluated with multiple tools [Samtools Stats (Li *et al.*, 2009), Qualimap (Okonechnikov *et al.*, 2016) and Deeptools (Ramirez *et al.*, 2014)]. The mapped reads will then be translated into a quantitative measure of gene expression using HTSeq (Anders *et al.*, 2015). The differential gene expression between the pregnant and non-pregnant samples will be analysed with the DESeq2 package, which fits generalized linear models for each gene and compares the logarithmic fold changes adjusting for multiple testing as described in detail elsewhere (Love *et al.*, 2014).

Statistical analysis

Sample size calculation and feasibility

We performed a sample size calculation using PASS® version 11.0 (NCSS). As previously stated, most of the trials thus far have 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 proceeded with the calculation of the adequate sample size needed to detect an increase of 10% (from 32 to 42%) in the intervention group using a two-side Fischer's exact test with a significance level (alpha) of 0.05. To achieve an 80% power using a 1:1 randomization ratio, each group would need at least 180 subjects, adding up to a total of 360 subjects required for this trial.

We also performed an additional sample size calculation for the transcriptomic expression analysis to confirm that the trial would be adequately powered for this secondary outcome. We estimated that endometrium exogenously stimulated would have a differential gene expression (>2-fold variation) after a conservative evaluation of at least 20 million reads/counts. Using the algorithm and maximal human biologic variation estimates proposed by Hart *et al.* (2013), we concluded that at least 36 patients would be required to achieve a 90% power to detect the expected difference using a false-positive detection rate of 0.01 and coefficient of variation of 0.74.

The centre undertaking this clinical trial performs over 5000 oocyte retrievals per year, with ~20 patients per month being eligible for the study. Assuming a conservative participation rate of 40–50%, we estimate that we will require between 36 and 45 months to conclude this trial.

Analysis of clinical outcomes

Continuous baseline patient and cycle characteristics will be detailed using descriptive measures of centre and spread. Specifically, normally distributed data will be presented using mean and standard deviation, while non-normal continuous data will be summarized using median and interquartile range. Categorical data will be presented using absolute and relative within-group frequencies. The reporting of data will be done in accordance to CONSORT guidance (Schulz *et al.*, 2010).

All primary and secondary dichotomous outcomes will be compared among the treatment groups using the χ^2 test. All continuous outcomes will be compared with either the *t*-test or Mann–Whitney test depending

on the normality of their distribution. The primary analysis will be performed according to the intention-to-treat (ITT) principle. However, a PP analysis, as mentioned before, will also be considered.

All tests will be two-sided with a *P*-value being considered significant whenever below 0.05. For the statistical analysis, we will use Stata Software® version 13.1 (StataCorp).

Missing outcome data

Missing efficacy and safety outcomes will be considered as negative, regardless of the cause which may justify the lack of said information (e.g. cycle cancelled due to the development of no embryo or loss to follow-up). These patients will be included in the analysis following the ITT principle.

Data management, monitoring and dissemination of results

Data will be collected in a secure and encrypted eCRF created specifically for the trial using Filemaker Pro® version 13 (Filemaker Inc.) hosted on a dedicated server at the *CRG-UZ Brussel*. The database has inbuilt validation procedures to ensure the correct introduction of data and avoid cases of missing information where such is not applicable. The doctors, study nurses and research assistants collaborating in the trial will be responsible for the data collection. Data will be stored for at least 20 years.

No specific data safety monitoring board will be established for this trial. However, the UZ Brussel has both (i) a formal structure for the systematic reporting and auditing of adverse events and (ii) a Clinical Trial Centre, responsible for the regular monitoring and auditing of ongoing trials. These quality assurance measures have been accredited by the Joint Commission International (JCI) and have NEN ISO 15224:2012 certification.

The results of the study will be publicly disseminated following submission in peer-reviewed scientific journals.

Ethics and quality assurance

This study has received ethical approval by the Ethical Committee of the UZ Brussel.

The center performing this clinical trial is fully accredited by the Association for the Accreditation of Human Research Protection Programme (AAHRPP).

Discussion

Despite the weak biological plausibility and lack of knowledge regarding the potential risks associated with endometrial scratching (Simon and Bellver, 2014), this procedure is still widely applied in current clinical practice (Lensen *et al.*, 2016). This clinical trial aims to pragmatically assess the potential benefits and harms of a generalized use of this strategy. Furthermore, we will evaluate whether we are able to adequately predict ER while circumventing the need for endometrial injury during the period of the window of implantation currently required for the ER testing. Since, with the strategy applied in this clinical trial, the sampling procedure is performed during the proliferative phase, ER can be assessed while the uterine lining recovers from the short-term injury, prior to the window of implantation (Zhou *et al.*, 2008) and within the same cycle as embryo transfer. This novel approach has great clinical significance, since it may allow physicians, for the first time, to adequately assess ER during the same treatment cycle and better tailor the timing of embryo transfer. This would contribute substantially to the reduction of the 'role of chance' in ART and may finally eliminate the inevitable blind embryo transfer of top quality embryos into non-receptive uteri, ultimately resulting in higher pregnancy rates per embryo transfer. As the prevalence of delayed childbearing increases, such a development would be of significance for the over 1 million couples annually spending up to 50% of their annual income on ART in an attempt to become parents (Connolly *et al.*, 2010).

Despite the strengths of this study, there are two potential limitations that need to be addressed. First, our power calculation was based on CPR, given the fact that this was the most studied outcome in published data at the time of the design of the trial (Nastri *et al.*, 2012). In order to tackle this potential limitation, we decided to calculate our sample size more conservatively, using a 10% expected difference in terms of CPR while maintaining the follow-up of our sample until live birth. Since then, an updated meta-analysis (Nastri *et al.*, 2015) has reported more robust live-birth outcomes and estimated a potential benefit of endometrial scratching in terms of LBR of ~12% (from 26 to 34%). If confirmed in our RCT, this will imply that our sample will have at least 80% power to detect this estimated difference as well. Furthermore, given the lack of previous robust information on the potential adverse events related to intentional endometrial injury (Simon and Bellver, 2014), we were unable to adequately calculate the necessary sample for these secondary outcomes. For this reason, it is possible that this trial may eventually be underpowered to adequately detect differences in terms of adverse events.

Supplementary data

Supplementary data are available at *Human Reproduction Open* online.

Authors' roles

S.S.R. and D.S. are the principal investigators of the trial. D.S. is the research team head. S.S.R., D.S. and S.M. participated in the initial conception and design of the clinical trial. S.S.R. and C.B. wrote the first draft of the protocol. S.S.R., H.T., S.M. and C.B. contributed to the editing of the final version of the protocol. S.M. is responsible for the processing and analysis of all biopsy samples collected. All authors are involved in the subject recruitment process. All authors read and approved the final version of the protocol.

Funding

Research Foundation—Flanders (FWO, 1524417N). This organization has no further role in the study, namely with regards to protocol development, study conduction and evaluation of results.

Conflict of interest

The authors have no conflicts of interest to declare.

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