**Human Reproduction, Vol.29, No.11 pp. 2474–2481, 2014**
Advanced Access publication on September 8, 2014 doi:10.1093/humrep/deu213

human reproduction

ORIGINAL ARTICLE *Infertility*

# The effect of endometrial injury on ongoing pregnancy rate in unselected subfertile women undergoing *in vitro* fertilization: a randomized controlled trial

**Tracy Wing Yee Yeung*, Joyce Chai, Raymond Hang Wun Li, Vivian Chi Yan Lee, Pak Chung Ho, and Ernest Hung Yu Ng**

Department of Obstetrics & Gynecology, The University of Hong Kong, Hong Kong

*Correspondence address. Department of Obstetrics & Gynecology, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong. Tel: +852-22553374; Fax: +852-28175374; E-mail: tracyyeungwy@gmail.com

*Submitted on April 17, 2014; resubmitted on June 26, 2014; accepted on July 21, 2014*

**STUDY QUESTION:** Does endometrial injury in the cycle preceding ovarian stimulation for *in vitro* fertilization (IVF) improve the ongoing pregnancy rate in unselected subfertile women?

**SUMMARY ANSWER:** Endometrial injury induced by endometrial aspiration in the preceding cycle does not improve the ongoing pregnancy rate in unselected subfertile women undergoing IVF.

**WHAT IS KNOWN ALREADY:** Implantation failure remains one of the major limiting factors for IVF success. Mechanical endometrial injury in the cycle preceding ovarian stimulation of IVF treatment has been shown to improve implantation and pregnancy rates in women with repeated implantation failures. There is limited data on unselected subfertile women, especially those undergoing their first IVF treatment.

**STUDY DESIGN, SIZE, DURATION:** This randomized controlled trial recruited 300 unselected subfertile women scheduled for IVF/ICSI treatment between March 2011 and August 2013. Subjects were randomized into endometrial aspiration (EA) ($n = 150$) and non-EA ($n = 150$) groups according to a computer-generated randomization list.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** Subjects were recruited and randomized in the assisted reproductive unit at the University of Hong Kong. In the preceding cycle, women in the EA group underwent endometrial aspiration using a Pipelle catheter in mid-luteal phase. All women were treated with a cycle of IVF/ICSI. Pregnancy outcomes were compared.

**MAIN RESULTS AND THE ROLE OF CHANCE:** There were no significant differences in baseline or cycle characteristics between the groups. There were 209 subjects (69.7%) who were undergoing their first IVF cycle and 91 (30.3%) subjects who had repeated cycles. There was no significant difference in ongoing pregnancy rates [26.7% (40/150) versus 32.0% (48/150); RR 0.833 (95% CI 0.585–1.187), $P = 0.375$] in the EA and non-EA groups. The implantation rates [32.8% (67/204) versus 29.7% (68/229); RR 1.080 (95% CI 0.804–1.450), $P = 0.120$], clinical pregnancy rates [34.0% (51/150) versus 38.0 (57/150); RR 0.895 (95% CI 0.661–1.211), $P = 0.548$], miscarriage rates [30.3% (17/56) versus 18.6% (11/59), RR 1.628 (95% CI 0.838–3.164), $P = 0.150$] and multiple pregnancy rates [31.3% (16/51) versus 19.3% (11/57), RR 1.626 (95% CI 0.833–3.172), $P = 0.154$] were all comparable between the EA and non-EA groups. Subgroup analysis in women having first embryo transfer ($n = 209$) also demonstrated no significant difference in ongoing pregnancy rates, but for women undergoing repeated cycles ($n = 91$), the ongoing pregnancy rate was significantly lower in the EA group than in the non-EA group.

**LIMITATIONS, REASONS FOR CAUTION:** The study aimed at assessing an unselected population of subfertile women by recruiting consecutive women attending our fertility clinic. However, since the majority of the recruited women (69.7%) were having their first IVF treatments, the results may not be generalizable to all women undergoing IVF.

**WIDER IMPLICATIONS OF THE FINDINGS:** Previous RCTs and meta-analyses have suggested improved pregnancy rates after pretreatment endometrial injury in women with repeated implantation failure. A recent RCT also showed increased pregnancy rates in unselected

subfertile women after endometrial injury, although that study was terminated early and thus underpowered. Our study showed with adequate power that no significant improvement in pregnancy rates was observed after endometrial injury in unselected women undergoing IVF treatment.

**STUDY FUNDING/COMPETING INTEREST(S):** The study was supported by the Small Project Funding 201309176012 of the Committee on Research and Conference Grants, University of Hong Kong. The authors have nothing to disclose.

**TRIAL REGISTRATION NUMBER:** HKCTR-1646 and NCT 01977976.

**Key words:** endometrial injury / endometrium / *in vitro* fertilization / embryo transfer / pregnancy rate

# Introduction

Implantation failure remains one of the major factors limiting success in *in vitro* fertilization (IVF) treatment. According to the ESHRE data on assisted reproductive technology outcomes across Europe in 2009, only 32% of fresh embryo transfers resulted in clinical pregnancies (Ferraretti *et al.*, 2013). Implantation requires a precise crosstalk between the embryo and the endometrium, and the exact mechanism remains largely unknown. Even with the introduction of preimplantation genetic screening (PGS) and replacement of chromosomally normal embryos, successful implantation cannot be guaranteed. Data from the ESHRE PGD Consortium 2009/10 reported implantation rates of 22.6% for all women undergoing PGS and 23.9% for (Barash *et al.*, 2003) women with repeated implantation failure (RIF) (Moutou *et al.*, 2014). It is apparent that the endometrium or the interaction between embryos and the endometrium has an important role in achieving implantation.

Local injury to the endometrium has been proposed as a means to improve implantation in women with RIF. Initial non-randomized studies showed a doubling of implantation rates after 2–4 endometrial injuries performed at different time points of the menstrual cycle in women with previous implantation failure (Barash *et al.*, 2003; Raziel *et al.*, 2007). Following that, a number of randomized trials focusing on women with RIF have been conducted. The majority of the trials have demonstrated significant improvements in implantation rates, clinical pregnancy rates and/or live birth rates following endometrial injury performed in the preceding cycle (Karimzadeh *et al.*, 2009; Narvekar *et al.*, 2010; Gibreel *et al.*, 2013), while another small trial failed to detect any benefit (Baum *et al.*, 2012). Recent systematic reviews and meta-analyses, based mainly on non-randomized or unpublished studies, have concluded the beneficial effect of endometrial injury in women undergoing IVF (El-Toukhy *et al.*, 2012; Potdar *et al.*, 2012). However, the quality of the included trials has been criticized (Simón and Bellver, 2014) and there are limited data supporting the use of endometrial injury in unselected women undergoing IVF.

The aim of our study was to assess whether endometrial injury in the cycle preceding ovarian stimulation can improve the ongoing pregnancy rates in unselected subfertile women undergoing IVF, including those undergoing their first IVF treatment.

# Materials and Methods

## Study design and participants

This randomized controlled trial was conducted between March 2011 and October 2013 in the Assisted Reproduction Unit at the Department of Obstetrics and Gynaecology, University of Hong Kong. Consecutive women attending the unit and indicated for IVF treatment were screened and recruited.

The inclusion criteria include: (i) subfertile women indicated for IVF treatment and (ii) a normal uterine cavity demonstrated by saline infusion sonogram (SIS) or hysteroscopy. Women were excluded from recruitment due to: (i) the presence of an endometrial polyp or fibroid distorting the uterine cavity; (ii) the presence of hydrosalpinx; (iii) IVF treatment carried out for preimplantation genetic diagnosis; or (iv) the use of donor oocytes.

Eligible women were fully counselled and written consent was obtained. The study had been approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster and was registered under the Hong Kong Clinical Trial Center (HKCTR-1646) and Clinicaltrials.gov (NCT01977976).

## Randomization and blinding

Recruited subjects were randomized into the EA or non-EA groups in a 1 to 1 ratio according to a computer-generated randomization list with blocks of 10 in sealed envelopes by a research nurse not involved in the clinical management of the subjects. Due to the nature of the intervention, the clinician performing the endometrial aspirate and the patients were not blinded.

## Endometrial injury

For subjects in the EA group, endometrial aspirate was performed 7 days after the LH surge in ovulatory women or on cycle Day 21 in anovulatory women in the cycle immediately preceding the scheduled IVF treatment. The LH surge was defined as an elevation of serum LH to $\geq$2 times the average of the previous 3 days with an absolute level of $\geq$20 IU/l. Subjects were instructed to use non-hormonal means of contraception during that cycle. The procedure was performed in a standard approach using a Pipelle catheter (Pipelle de Cornier, Laboratoire C.C.D., France). The Pipelle catheter was introduced through the cervix up to the uterine fundus. The piston was drawn back to the end of the sheath to create a negative pressure. The sheath was rotated and moved back and forth between the fundus and internal os at least 3–4 times before it was gently withdrawn to ensure endometrial tissue has been obtained. Those in the non-EA group received the usual care.

## Ovarian stimulation and IVF

All patients started their IVF treatment in the subsequent cycle with ovarian stimulation using either the long or fixed antagonist protocols as previously descried (Li *et al.*, 2013). On Day 2–3 on the menstrual cycle, women underwent transvaginal ultrasound examination and serum estradiol measurement. Human menopausal gonadotrophin (hMG) (Menogon, Ferring GmbH, Kiel, Germany) or recombinant FSH (Puregon, Organon, Dublin, Ireland or Gonal F, Merck Serono S.p.A, Modugno, Italy) were started with a starting dose between 150 and 300 IU per day based on the antral follicle count, age and previous ovarian response, according to the standard operation procedures. Ovarian response was monitored by serial transvaginal

scanning with or without hormonal monitoring. Further dosage adjustments were based on the ovarian response. Gonadotrophin releasing hormone (GnRH) antagonist 0.25 mg/day (Orgalutran, Organon, Dublin, Ireland) was started on the sixth day of stimulation. When one to three leading follicles were ≥18 mm, 5000–10 000 IU human chorionic gonadotrophin (hCG, Pregnyl [Organon, Oss, the Netherlands]) or 250 μg ovidrel (Merck Serono S.p.A, Modugno, Italy) was given to trigger final maturation of the oocytes. Transvaginal ultrasound guided oocyte retrievals (TUGOR) were scheduled 36 h later. A maximum of two embryos were transferred 2 days after TUGOR. Excess good quality embryos were frozen for subsequent transfer.

## Outcome measures

The primary outcome was the ongoing pregnancy rate. Secondary outcomes included the implantation rate, clinical pregnancy rate, multiple pregnancy rate and miscarriage rate.

Ongoing pregnancy was defined as the presence of at least one fetal heart pulsation on ultrasound beyond 20 weeks. Clinical pregnancy was defined as the presence of at least one gestational sac on ultrasound at 6 weeks. Implantation rate was the number of sacs detected on ultrasound divided by the number of embryos transferred. The miscarriage rate was defined as the number of miscarriages before 20 weeks divided by the number of women

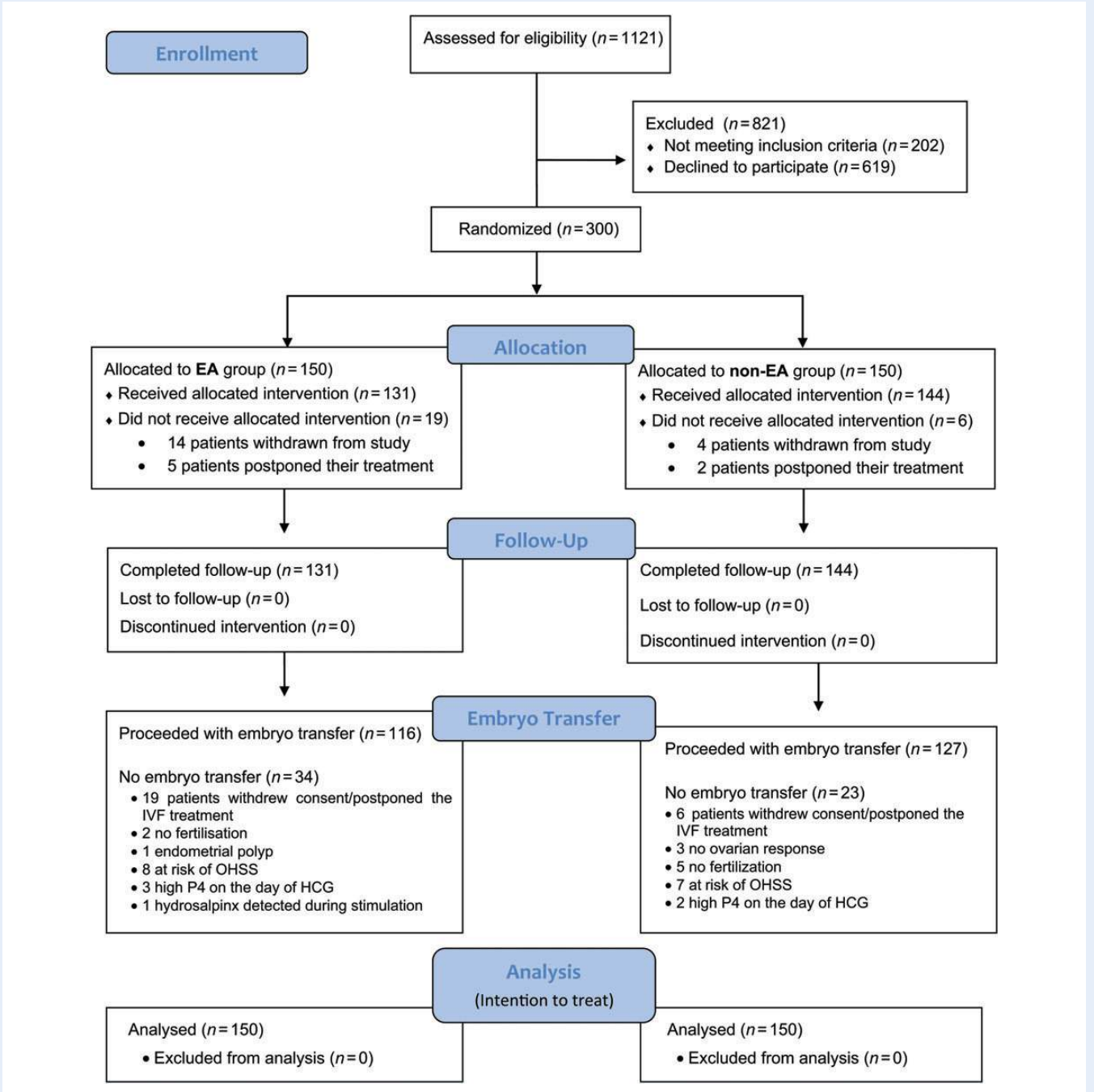


**Figure 1** CONSORT 2010 flow diagram.

with a positive pregnancy test. Multiple pregnancy was defined as a pregnancy with more than one gestational sac detected on ultrasound at 6 weeks.

### Sample size calculation

The ongoing pregnancy rate of IVF treatment in our ART unit in 2009 was 32% per cycle started. Previous controlled studies demonstrated a doubling of implantation and clinical pregnancy rates after endometrial injury by endometrial biopsy (Barash *et al.*, 2003; Raziel *et al.*, 2007; Zhou *et al.*, 2008). Assuming there is a 50% increase in ongoing pregnancy rate (i.e. increased from 32 to 48%) in the study group, 146 subjects are required in each arm to give a test of significance of 0.05 and a power of 0.8. Therefore 300 subjects were recruited in the RCT to allow for some drop-outs.

### Statistics

The analysis was performed based on the intention-to-treat (ITT) and per protocol principles. Statistical comparisons were carried out using Mann–Whitney *U*-test, Chi-square test, Fisher's exact test and multivariate regression analysis where appropriate with the Statistical Program for Social Sciences (SPSS, Inc., Version 21.0, Chicago, IL, USA). A two-sided $P < 0.05$ was taken as statistically significant.

## Results

### Participant flow

Between March 2011 and August 2013, a total of 300 subjects were recruited (Fig. 1 – Consort 2010 Flow Diagram). The main reason for refusal was because of the inconvenience of additional clinic visits and the anticipated discomfort associated with blood taking and the endometrial aspirate.

### Baseline and cycle characteristics

Baseline characteristics of the EA and non-EA groups including the age of women, body mass index, duration, type and causes of subfertility and number of previous IVF cycles were represented in Table I. No significant differences were detected in the cycle characteristics including the stimulation protocol, the number of embryos transferred and quality of the embryo transferred as shown in Table II.

### Primary outcomes

There was no significant difference in the ongoing pregnancy rates between the EA group and non-EA group based on both ITT [26.7% (40/150) versus 32.0% (48/150); RR 0.833 (95% CI = 0.585–1.187), $P = 0.375$] and per protocol [34.5% (40/116) versus 37.8% (48/127), RR 0.912 (95% CI = 0.652–1.276), $P = 0.596$] analyses. Upon multivariate logistic regression analysis, female age was the only factor significantly affecting the ongoing pregnancy rate [B = −0.145, OR 0.865 (95% CI = 0.789–0.948), $P = 0.002$].

### Secondary outcomes

There were no significant differences in the implantation, clinical pregnancy, live birth, multiple pregnancy and miscarriage rates based on both the ITT and per protocol analyses (Table III).

The endometrial aspirations were successful in all attempted subjects. No complications, including excessive bleeding, significant pain requiring intervention or acute pelvic infection, were reported from the aspiration procedure throughout the study period.

**Table I Baseline characteristics of the EA and non-EA groups.**

| | EA group ($n = 150$) | Non-EA ($n = 150$) |
|---|---|---|
| Age (years) | 36 (34–38) | 37 (34–38) |
| BMI (kg/m$^2$) | 21.2 (20.1–22.9) | 22.2 (20.3–24.4) |
| Smoking | | |
| Yes | 12 (8.0%) | 13 (8.7%) |
| No | 133 (88.7%) | 128 (85.3%) |
| Ex-smoker | 5 (3.3%) | 9 (6.0%) |
| Menstrual cycles | | |
| Ovulatory | 119 (79.3%) | 124 (82.7%) |
| Anovulatory | 31 (20.7%) | 26 (17.3%) |
| Duration of subfertility (years) | 4.0 (2.0–6.6) | 4.0 (2.0–6.0) |
| Type of subfertility | | |
| Primary | 94 (62.7%) | 87 (58.0%) |
| Secondary | 56 (37.3%) | 63 (42.0%) |
| Causes of subfertility | | |
| Tuboperitoneal | 27 (18.0%) | 24 (16.0%) |
| Male | 50 (33.3%) | 60 (40.0%) |
| Unexplained | 33 (22.0%) | 35 (23.3%) |
| Endometriosis | 8 (5.3%) | 8 (5.3%) |
| Mixed | 32 (21.4%) | 23 (15.4%) |
| Number of previous transfer(s) | | |
| 0 | 104 (69.3%) | 105 (70.0%) |
| 1–2 | 31 (20.7%) | 31 (20.7%) |
| ≥3 | 15 (10.0%) | 14 (9.3%) |

Data expressed as median (25th to 75th centiles) or number (percentage) as appropriate.
BMI, body mass index.

### Subgroup analyses

Subgroup analysis was performed by stratifying women into those undergoing their first or repeated IVF cycles (i.e. with at least one fresh or frozen-thawed embryo transfer before).

There were no significant differences in the ongoing pregnancy rates between the EA group and non-EA group for women undergoing their first IVF cycles based on either ITT or per protocol analyses. For women undergoing their repeated cycles, significantly lower ongoing pregnancy and live birth rates were noted in the EA group based on both the ITT and per protocol analyses (Table IV).

Multivariate logistic regression analysis revealed female age being the only significant factor affecting the ongoing pregnancy rate in women undergoing first cycle of IVF [B = −0.121, OR = 0.886 (95% CI = 0.799–0.983), $P = 0.022$]; while the number of top quality embryo transferred was the only significant factor in women having repeated cycles of IVF [B = 0.884, OR = 2.421 (95% CI = 1.053–5.566), $P = 0.037$]

There were also no significant differences in the ongoing pregnancy rates after endometrial injury among subjects who were ovulatory or anovulatory [33.1% (41/124) versus 26.9% (7/19), RR 1.277 (0.576–2.831), $P = 0.647$].

**Table II Cycle characteristics of the EA and non-EA groups proceeding to ovarian stimulation.**

| | EA group ($n$ = 131) | Non-EA group ($n$ = 144) | $P$-value |
|---|---|---|---|
| Protocol | | | 0.397 |
| Long agonist | 69/131 (52.7%) | 68/144 (47.2%) | |
| Antagonist | 62/131 (47.3%) | 76/144 (52.8%) | |
| Insemination | | | 0.397 |
| Conventional | 88/131 (67.2%) | 89/144 (61.8%) | |
| ICSI | 43/131 (32.8%) | 55/144 (38.2%) | |
| Total gonadotrophin used (IU) | 2225 (1650–3000) | 2400 (1800–3000) | 0.285 |
| Endometrial thickness on the day on HCG (mm) | 11.9 (10.0–13.7) | 11.7 (10.2–13.3) | 0.676 |
| Number of oocytes obtained | 8 (5–12) | 8 (5–12.25) | 0.933 |
| Number of fertilized embryos | 5 (3–9) | 5 (3–8) | 0.632 |
| Total number of TQEs | 1 (0–3) | 1 (0–3) | 0.829 |
| Number of transferred embryos | | | 0.684 |
| 0 | 16 (12.2%) | 17 (11.8%) | |
| 1 | 27 (20.6%) | 25 (17.4%) | |
| 2 | 88 (67.2%) | 102 (70.8%) | |
| Number of TQE transferred at fresh cycle | | | |
| 0 | 34 (29.8%) | 36 (28.8%) | 0.850 |
| 1 | 42 (36.8%) | 43 (34.4%) | |
| 2 | 38 (33.3%) | 46 (36.8%) | |
| Day of embryo transfer | | | 0.717 |
| 2 | 114 (87.0%) | 125 (86.6%) | |
| 3 | 1 (0.8%) | 0 (0%) | |
| 5 | 1 (0.8%) | 2 (1.4%) | |

TQE, top quality embryo.

**Table III Pregnancy outcomes of EA and non-EA groups (ITT and per protocol analyses).**

| **Analysis by ITT ($n$ = 300)** | | | | |
|---|---|---|---|---|
| | **EA group ($n$ = 150)** | **Non-EA group ($n$ = 150)** | **Relative risk (95% CI)** | ***P*-value** |
| Ongoing pregnancy rate | 40/150 (26.7%) | 48/150 (32.0%) | 0.833 (0.585–1.187) | 0.375 |
| Live birth rate | 39/150 (26.0%) | 48/150 (32.0%) | 0.813 (0.569–1.161) | 0.254 |
| Implantation rate | 67/204 (32.8%) | 68/229 (29.7%) | 1.080 (0.804–1.450) | 0.120 |
| Clinical pregnancy rate | 51/150 (34.0%) | 57/150 (38.0%) | 0.895 (0.661–1.211) | 0.548 |
| Miscarriage rate | 17/56 (30.3%) | 11/59 (18.6%) | 1.628 (0.838–3.164) | 0.150 |
| Multiple pregnancy rate | 16/51 (31.3%) | 11/57 (19.3%) | 1.626 (0.833–3.172) | 0.154 |
| **Analysis per protocol ($n$ = 243)** | | | | |
| | **EA group ($n$ = 116)** | **Non-EA group ($n$ = 127)** | **Relative risk (95% CI)** | ***P*-value** |
| Ongoing pregnancy rate | 40/116 (34.5%) | 48/127 (37.8%) | 0.912 (0.652–1.276) | 0.596 |
| Live birth rate | 39/116 (33.6%) | 48/127 (37.8%) | 0.890 (0.634–1.490) | 0.499 |
| Implantation rate | 67/204 (32.8%) | 68/229 (29.7%) | 1.080 (0.804–1.450) | 0.120 |
| Clinical pregnancy rate | 51/116 (44.0%) | 57/127 (44.9%) | 0.980 (0.739–1.298) | 0.898 |
| Miscarriage rate | 17/56 (30.3%) | 11/59 (18.6%) | 1.532 (0.780–3.010) | 0.272 |
| Multiple pregnancy rate | 16/51 (31.3%) | 11/57 (19.3%) | 1.626 (0.833–3.172) | 0.154 |

**Table IV Subgroup analysis: pregnancy outcomes among subjects in first or repeated IVF cycles (ITT and per protocol analyses).**

| **First cycle** | | | | |
|---|---|---|---|---|
| | **EA group** | **Non-EA group** | **RR (95% CI)** | ***P*-value** |
| Analysis by ITT ($n = 209$) | | | | |
| Ongoing pregnancy rate | 33/105 (31.4%) | 32/104 (30.8%) | 1.021 (0.682–1.529) | 1.000 |
| Clinical pregnancy rate | 42/105 (40.0%) | 38/104 (36.5%) | 1.095 (0.775–1.546) | 0.670 |
| Live birth rate | 32/105 (30.5%) | 32/104 (30.8%) | 0.991 (0.659–1.490) | 0.963 |
| Analysis per protocol ($n = 162$) | | | | |
| Ongoing pregnancy rate | 33/76 (43.4%) | 32/86 (37.2%) | 1.167 (0.801–1.699) | 0.428 |
| Clinical pregnancy rate | 42/76 (55.3%) | 38/86 (44.2%) | 1.251 (0.915–1.709) | 0.208 |
| Live birth rate | 32/76 (42.1%) | 32/86 (37.2%) | 1.132 (0.773–1.656) | 0.524 |
| Implantation rate | 55/133 (41.4%) | 46/155 (29.6%) | 1.278 (0.912–1.791) | 0.270 |
| Multiple pregnancy rate | 13/42 (31.0%) | 8/38 (21.1%) | 1.470 (0.685–3.155) | 0.323 |
| Miscarriage rate | 13/45 (28.9%) | 7/39 (17.9%) | 1.610 (0.714–3.628) | 0.251 |
| **Repeated cycle** | | | | |
| | **EA group** | **Non-EA group** | **RR (95% CI)** | ***P*-value** |
| Analysis by ITT ($n = 91$) | | | | |
| Ongoing pregnancy rate | 7/45 (15.6%) | 16/46 (34.8%) | 0.447 (0.203–0.983)* | 0.045* |
| Clinical pregnancy rate | 9/45 (20%) | 18/46 (39.1%) | 0.511 (0.257–1.016) | 0.055 |
| Live birth rate | 6/45 (13.3%) | 15/46 (32.6%) | 0.409 (0.174–0.960)* | 0.040* |
| Analysis per protocol ($n = 81$) | | | | |
| Ongoing pregnancy rate | 7/40 (17.5%) | 16/41 (39.0%) | 0.448 (0.207–0.973)* | 0.042* |
| Clinical pregnancy rate | 9/40 (22.5%) | 18/41 (43.9%) | 0.513 (0.261–1.003) | 0.051 |
| Live birth rate | 6/40 (15.0%) | 15/41 (36.6%) | 0.432 (0.187–0.997)* | 0.049* |
| Implantation rate | 12/71 (16.9%) | 22/74 (29.7%) | 0.631 (0.333–1.195) | 0.468 |
| Multiple pregnancy rate | 3/9 (33.3%) | 3/19 (15.8%) | 2.111 (0.525–8.476) | 0.292 |
| Miscarriage rate | 4/11 (36.4%) | 4/20 (20.0%) | 1.818 (0.562–5.885) | 0.318 |

*Denotes statistically significant difference.

# Discussion

From the present study, endometrial injury induced by endometrial aspiration in the preceding cycle did not result in significant improvement in the ongoing pregnancy rate among unselected subfertile women undergoing IVF. No significant improvement could be demonstrated when the analysis was limited in women undergoing their first IVF cycles, but a significantly lower ongoing pregnancy rate with endometrial injury was detected in those undergoing repeated IVF cycles.

Endometrial injury in the cycle prior to ovarian stimulation in IVF has been reported to result in improved clinical pregnancy and/or live birth rates (Karimzadeh *et al.*, 2009; Narvekar *et al.*, 2010; Gibreel *et al.*, 2013; Shohayeb and El-Khayat, 2012). It has been postulated that the local injury to endometrium induces secretions of cytokines and growth factors that will stay in the basal layer of endometrium for a few cycles and enhance decidualisation and facilitate implantation (Finn and Martin, 1972; Sharkey, 1998; Akita *et al.*, 2000; Basak *et al.*, 2002). Endometrial injury has also been shown to up-regulate the gene expression related to endometrial receptivity (Kalma *et al.*, 2009) which optimizes endometrial development (Zhou *et al.*, 2008; Almog *et al.*, 2010).

Previous studies have typically reported a 2-fold increase in clinical pregnancy and/or live birth rates after endometrial injury. The reported significant benefits in women with RIF have made it a tempting intervention to be offered to all women prior to their IVF treatments. However, most of the studies have been underpowered and there has been very limited data exploring the role of EA in unselected subfertile women. One of the major strengths of our study was the ability to assess the effect of routine endometrial injury with adequate power, and provide clinicians with evidence regarding the value of endometrial injury in routine fertility practice.

In contrast with previous results, our subgroup of women who had at least one failed transfer showed reduced ongoing pregnancy and live birth rates. Although this subgroup of women who had at least one previous

failed transfer in our present study is relatively small and did not represent any particular clinical entity, with a range of previous failed transfers from one to six, we cannot exclude any potential harm of endometrial injury even when it is performed in the luteal phase prior to ovarian stimulation. We agree that women having genuine RIF may represent a distinct clinical entity with different fertility potential, where endometrial injury may have a role in improving endometrial receptivity and thus implantation and pregnancy outcomes. However, our present study was not targeted to address this and the subgroup of patients having $\geq 3$ previous transfers was too small ($n = 27$) to draw any reliable conclusion. Further studies employing a standard definition of RIF would be required for guide management in this difficult subgroup.

It is possible that the timing and number of endometrial biopsies, as well as the degree of injury, may have an impact on the pregnancy outcomes. There is no consensus on the optimal timing and the number of procedure(s) required for the endometrial injury to exert its maximal effect, although a detrimental effect has been reported when the endometrial injury was performed in the transfer cycle on the day of oocyte retrieval (Karimzade *et al.*, 2010). In the present study, we performed a single endometrial biopsy in the cycle prior to IVF during the mid-luteal phase in ovulatory women. This is the presumed 'window of implantation' with the highest abundance of cytokines, growth factors and monocytes cells in the endometrium (Gnainsky *et al.*, 2010), where the effect of endometrial injury, if any, may be maximized.

It is also of note that although a couple of recent systematic reviews and meta-analyses have concluded a beneficial effect of endometrial injury in women with RIF (El-Toukhy *et al.*, 2012; Potdar *et al.*, 2012), they have included non-randomized studies and only a limited number of the available randomized trials were included. Only one of the included studies reported the live birth rate and showed a marginal improvement. Indeed when we reviewed all the available RCTs assessing the effect of endometrial injury on pregnancy rates, most of them either did not have an *a priori* sample size calculation or a well-defined primary outcome (Karimzadeh *et al.*, 2009; Narvekar *et al.*, 2010; Baum *et al.*, 2012; Gibreel *et al.*, 2013), or they were prematurely terminated before completion of recruitment (Nastri *et al.*, 2013). These factors would have limited the ability to draw reliable conclusions with adequate power.

A recent publication by Nastri *et al.* and including 158 women reported a significantly improved clinical pregnancy rate of 49.4% after endometrial biopsy using a Pipelle catheter in the preceding luteal phase, compared with a rate of 29.1% in the control group ($P = 0.01$) among unselected women undergoing IVF (Nastri *et al.*, 2013). Although the subject inclusion criteria and intervention were essentially the same as in our study, almost 90% of subjects in that study had previous unsuccessful embryo transfers, compared with >70% of treatment naïve women in our trial. The differences in subject characteristics may account for the discrepancies of the results; and the fact that the previous study had been prematurely terminated after interim analysis may have overestimated the treatment effect leading to biased results.

One of the limitations of our study was the absence of a placebo and both our clinicians and patients were not blinded to the randomization. However, owing to the nature of intervention, the clinicians could not be blinded and the patients would likely be aware of the intervention that they were allocated to even if a sham procedure in the vagina or cervix were performed. On the other hand, the primary outcome measure of ongoing pregnancy rate is an objective outcome that is unlikely to be subject to bias.

When performing the power calculation, we assumed a 50% increase in ongoing pregnancy rate to be clinically significant. Previous studies reporting improvement after endometrial injury typically reported a doubling of pregnancy rates. We adjusted it to a 50% increase as a more realistic estimation. It is possible that a smaller degree of improvement may not have been detected as significantly different and the results should therefore be interpreted with caution. Although the live birth rate was not used as the primary outcome measure in the power calculation, most pregnancies proceeding beyond 20 weeks resulted in live births and we have also presented the live birth data.

## Conclusion

Endometrial injury induced by an endometrial aspiration in the preceding cycle did not improve the ongoing pregnancy rate in unselected subfertile women undergoing IVF. Currently, there is lack of good evidence to support routine endometrial aspiration in unselected women prior to IVF treatment.

## Authors' roles

T.W.Y.Y. was involved in the study design, execution and analysis, manuscript drafting, critical discussion and final approval of the manuscript. E.H.Y.N. was involved in the study design and execution, critical discussion and final approval of the manuscript. J.C., V.C.Y.L., R.H.W.L., P.C.H. were involved in the study execution, critical discussion and final approval of the manuscript.

## Funding

The study was supported by the Small Project Funding 201309176012 of the Committee on Research and Conference Grants, University of Hong Kong.

## Conflict of interest

None declared.

## References

Akita S, Ishihara H, Abdur RM, Fujii T. Leukemia inhibitory factor gene improves skin allograft survival in the mouse model1. *Transplantation* 2000;**70**:1026–1031.

Almog B, Shalom-Paz E, Dufort D, Tulandi T. Promoting implantation by local injury to the endometrium. *Fertil Steril* 2010;**94**:2026–2029.

Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I. Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing *in vitro* fertilization. *Fertil Steril* 2003;**79**:1317–1322.

Basak S, Dubanchet S, Zourbas S, Chaouat G, Das C. Expression of pro-inflammatory cytokines in mouse blastocysts during implantation: modulation by steroid hormones. *Am J Reprod Immunol* 2002;**47**:2–11.

Baum M, Yerushalmi GM, Maman E, Kedem A, Machtinger R, Hourvitz A, Dor J. Does local injury to the endometrium before IVF cycle really affect treatment outcome? Results of a randomized placebo controlled trial. *Gynecol Endocrinol* 2012;**28**:933–936.

El-Toukhy T, Sunkara S, Khalaf Y. Local endometrial injury and IVF outcome: a systematic review and meta-analysis. *Reprod Biomed Online* 2012;**25**: 345–354.

Ferraretti A, Goossens V, Kupka M, Bhattacharya S, de Mouzon J, Castilla J, Erb K, Korsak V, Nyboe Andersen A. European IVF-Monitoring (EIM) Consortium for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2009: results generated from European registers by ESHRE. *Hum Reprod* 2013;**28**:2318–2331.

Finn C, Martin L. Endocrine control of the timing of endometrial sensitivity to a decidual stimulus. *Biol Reprod* 1972;**7**:82–86.

Gibreel A, Badawy A, El-Refai W, El-Adawi N. Endometrial scratching to improve pregnancy rate in couples with unexplained subfertility: a randomized controlled trial. *J Obstet Gynaecol Res* 2013;**39**:680–684.

Gnainsky Y, Granot I, Aldo PB, Barash A, Or Y, Schechtman E, Mor G, Dekel N. Local injury of the endometrium induces an inflammatory response that promotes successful implantation. *Fertil Steril* 2010;**94**:2030–2036.

Kalma Y, Granot I, Gnainsky Y, Or Y, Czernobilsky B, Dekel N, Barash A. Endometrial biopsy-induced gene modulation: first evidence for the expression of bladder-transmembranal uroplakin Ib in human endometrium. *Fertil Steril* 2009;**91**:1042–1049. e1049.

Karimzade MA, Oskouian H, Ahmadi S, Oskouian L. Local injury to the endometrium on the day of oocyte retrieval has a negative impact on implantation in assisted reproductive cycles: a randomized controlled trial. *Arch gynecol obstet* 2010;**281**:499–503.

Karimzadeh MA, Ayazi Rozbahani M, Tabibnejad N. Endometrial local injury improves the pregnancy rate among recurrent implantation failure patients undergoing *in vitro* fertilisation/intra cytoplasmic sperm injection: a randomised clinical trial. *Aust N Z J Obstet Gynaecol* 2009; **49**:677–680.

Li HWR, Lee VCY, Lau EYL, Yeung WSB, Ho PC, Ng EHY. Role of baseline antral follicle count and anti-Mullerian hormone in prediction of cumulative live birth in the first *in vitro* fertilisation cycle: a retrospective cohort analysis. *PLoS One* 2013;**8**:e61095.

Moutou C, Goossens V, Coonen E, De Rycke M, Kokkali G, Renwick P, Traeger-Synodinos J. ESHRE PGD Consortium data collection XII: cycles from January to December 2009 with pregnancy follow-up to October 2010. *Hum Reprod* 2014;**29**:880–903.

Narvekar SA, Gupta N, Shetty N, Kottur A, Srinivas M, Rao KA. Does local endometrial injury in the nontransfer cycle improve the IVF-ET outcome in the subsequent cycle in patients with previous unsuccessful IVF? A randomized controlled pilot study. *J Hum Reprod Sci* 2010;**3**:15.

Nastri CO, Ferriani RA, Raine-Fenning N, Martins WdP. Endometrial injury performed in the non-transfer cycle and assisted reproduction outcomes: a randomized controlled trial. *Ultrasound Obstet Gynecol* 2013;**42**:375–382.

Potdar N, Gelbaya T, Nardo LG. Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis. *Reprod Biomed Online* 2012;**25**:561–571.

Raziel A, Schachter M, Strassburger D, Bern O, Ron-El R, Friedler S. Favorable influence of local injury to the endometrium in intracytoplasmic sperm injection patients with high-order implantation failure. *Fertil Steril* 2007; **87**:198–201.

Sharkey A. Cytokines and implantation. *Rev Reprod* 1998;**3**:52–61.

Shohayeb A, El-Khayat W. Does a single endometrial biopsy regimen (S-EBR) improve ICSI outcome in patients with repeated implantation failure? A randomised controlled trial. *Eur J Obstet Gynecol Reprod Biol* 2012; **164**:176–179.

Simón C, Bellver J. Scratching beneath 'The Scratching Case': systematic reviews and meta-analyses, the back door for evidence-based medicine. *Hum Reprod* 2014;**29**:1618–1621.

Zhou L, Li R, Wang R, Huang H-x, Zhong K. Local injury to the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates. *Fertil Steril* 2008;**89**:1166–1176.