Reproductive BioMedicine Online (2012) **25**, 561–571

www.sciencedirect.com
www.rbmonline.com

REVIEW

Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis

Neelam Potdar [a,*], Tarek Gelbaya [b], Luciano G Nardo [c]

[a] *Leicester Fertility Centre, University of Leicester and University Hospitals of Leicester, Leicester, UK;* [b] *Leicester Fertility Centre, University Hospitals of Leicester, Leicester, UK;* [c] *Reproductive Medicine and Surgery Unit, Gynehealth, Manchester, UK*

[*] Corresponding author. *E-mail address:* neelam.potdar@yahoo.co.uk (N Potdar).

Neelam Potdar, MS, MD, MRCOG commenced her specialty training in the UK in 2001, thereafter joined research programme and obtained her MD degree from the University of Leicester in 2009. Since then she has been working as a subspecialist trainee in reproductive medicine and surgery and a clinical lecturer at the University of Leicester. Her research interests are implantation failure, lifestyle factors associated with subfertility and early pregnancy loss, and fertility preservation.

Abstract Mechanical endometrial injury (biopsy/scratch or hysteroscopy) in the cycle preceding ovarian stimulation for IVF has been proposed to improve implantation in women with unexplained recurrent implantation failure (RIF). This is a systematic review and meta-analysis of studies comparing the efficacy of endometrial injury versus no intervention in women with RIF undergoing IVF. All controlled studies of endometrial biopsy/scratch or hysteroscopy performed in the cycle preceding ovarian stimulation were included and the primary outcome measure was clinical pregnancy rate. Pooling of seven controlled studies (four randomized and three non-randomized), with 2062 participants, showed that local endometrial injury induced in the cycle preceding ovarian stimulation is 70% more likely to result in a clinical pregnancy as opposed to no intervention. There was no statistically significant heterogeneity in the methods used, clinical pregnancy rates being twice as high with biopsy/scratch (RR 2.32, 95% CI 1.72–3.13) as opposed to hysteroscopy (RR 1.51, 95% CI 1.30–1.75). The evidence is strongly in favour of inducing local endometrial injury in the preceding cycle of ovarian stimulation to improve pregnancy outcomes in women with unexplained RIF. However, large randomized studies are required before iatrogenic induction of local endometrial injury can be warranted in routine clinical practice.

KEYWORDS: endometrial scratch, hysteroscopy, ICSI, IVF, local endometrial injury, recurrent implantation failure

Introduction

Despite the escalating clinical and scientific advances in reproductive medicine, recurrent implantation failure (RIF) is a challenging and extremely disappointing problem faced by the clinicians and the couples alike. The term RIF has been used since 1983, different definitions have evolved and various investigations and treatment options have been

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2012.08.005

studied to improve pregnancy outcomes in this cohort (Margalioth et al., 2006). In women with unexplained RIF, despite good hormonal response, good-quality embryos, satisfactory endometrial development and no identifiable pathology, suboptimal endometrial receptivity is considered a key factor in inhibiting embryo implantation. During the implantation window, there is a cross-talk between the embryo and the endometrium to allow attachment, adhesion and invasion of the embryo. Morphologically, this pro-receptivity of the endometrium can be determined by stromal decidualization and the development of pinopodes and microvilli on the luminal epithelium (Dunn et al., 2003). At the molecular level, this is regulated by alteration in gene expression of cytokines, growth and transcription factors as well as adhesive molecules (Kalma et al., 2009; Paria et al., 2002). It has been shown that mechanical manipulation of the endometrium can enhance receptivity by modulating gene expression of factors required for implantation like glycodelin A (Mirkin et al., 2005), laminin alpha 4, integrin alpha 6 and matrix metalloproteinase 1 (Almog et al., 2010; Zhou et al., 2008).

The mechanical manipulation or local injury to the endometrium can be induced by endometrial biopsy (scratch) or hysteroscopy. Recently, in order to improve outcomes in women with unexplained RIF, various studies have examined pregnancy rates after inducing local endometrial injury in the cycle preceding ovarian stimulation. Because of the difference in the method used for inducing injury and the timing in relation to ovarian stimulation, there is considerable heterogeneity in the studies. A systematic review and meta-analysis of the available evidence was performed to assess the efficacy of endometrial injury (biopsy and/or hysteroscopy) as a treatment for unexplained RIF. The study has the potential to guide clinical practice for this challenging problem and direct future basic science and translational research.

Materials and methods

Literature search

Online searches of databases were performed in MEDLINE (1980–March 2012), EMBASE (1980–March 2012) and the Cochrane Library. The searches also included ISI Conference Proceedings and databases for registered and ongoing trials. A combination of medical subject headings and words were used to generate a subset of citations for: (i) local endometrial injury ('endometrial injury', 'local endometrial injury', 'endometrial scratch', 'endometrial biopsy' and 'endomet*'); (ii) hysteroscopy ('hysteroscopy', 'outpatient hysteroscopy' and 'hysterosco*'); (iii) RIF ('recurrent implantation failure', 'implantation failure' and 'failed cycle'); and (iv) outcomes after IVF and intracytoplasmic sperm injection (ICSI) ('outcome', 'IVF', '*in vitro* fertilization', 'intracytoplasmic sperm injection', 'ICSI' and 'assisted reproductive techniques'). These subsets were combined using 'AND' to generate final citations addressing the research question. The reference list of all published articles including review articles were examined to identify articles not noted by the electronic search of the databases. No language restrictions were placed on the searches, for all non-English articles of the relevant studies. The authors were contacted to obtain further information, as appropriate.

Study eligibility criteria

Initial selection of the studies was done if the studies compared intervention of endometrial injury (endometrial biopsy/scratch and/or hysteroscopy) in women undergoing IVF/ICSI treatment. The inclusion criteria for the studies were randomized and prospective non-randomized trials, population studied as women with RIF with exclusion of poor responders and intervention used in the cycle preceding ovarian stimulation. The studies were excluded if they were a retrospective study, for a first IVF/ICSI cycle or intervention was in the same cycle of ovarian stimulation or remote (more than a month before ovarian stimulation).

The included studies in the meta-analysis were randomized or prospective non-randomized, where women with previous failed implantation had undergone intervention of local endometrial injury compared with the matched control group in the cycle preceding ovarian stimulation. The non-randomized studies were vigorously reviewed and good-quality prospective trials that met all other predefined criteria were included. Exclusion of these studies would have led to missing out important data and available evidence.

Study selection and data extraction were performed by two authors (NP and TAG) independently; all articles including abstracts from the electronic searches were assessed and citations that met the initial predefined selection criteria were obtained. Trial quality assessment and final inclusion/exclusion decisions were made after examination of full manuscripts. After independent assessment of the manuscripts, any disagreement between the two reviewers was resolved by consultation with the third reviewer.

Data extraction

The selected studies were assessed for the methodological quality; for randomized studies, information was sought on the method of randomization, blinding, allocation concealment, intention-to-treat analysis and follow-up rate. For non-randomized studies, information was extracted as per the guidelines for meta-analysis of observational studies in epidemiology (MOOSE) (Stroup et al., 2000). For each study, information was obtained on the participants (number of previous failed IVF/ICSI cycles, ovarian response in the previous failed cycle, investigations for RIF and uterine cavity assessment), intervention used for endometrial injury (endometrial biopsy/scratch or hysteroscopy) and timing of intervention in relation to ovarian stimulation. Where there was doubt or lack of information, authors were contacted for further details.

Outcome measures

The primary outcome measure was clinical pregnancy rate (CPR). Live birth rate (LBR), implantation rate (IR), miscarriage and procedure-related complications were considered as secondary outcome measures.

Search results

The studies were selected and reported according to the PRISMA guidelines 2009 (Moher et al., 2009). A total of 279 citations were identified; 56 were selected in the initial screening and finally seven studies (four randomized controlled trials (RCT) and three non-randomized trials) were included in the meta-analysis (**Figure 1**). There were 49 citations excluded because they were review articles ($n = 12$) or case series ($n = 2$) or had outcomes not mentioned or timing of intervention different or unclear ($n = 10$) or a difference in the study populations ($n = 25$) (**Figure 1**).

Statistical analysis

Study features and outcomes were assembled in a tabular form, and formal meta-analysis was performed using Review Manager 5.1 (Cochrane Collaboration, 2001). A random-effect model (using Mantel-Haenszel method) was used because of difference in study designs and the method used for intervention (hysteroscopy and endometrial biopsy). The effect estimate was expressed as pooled risk ratio (RR) with 95% confidence interval (CI) and represented graphically by forest plots. Statistical heterogeneity was examined using chi-squared test and forest plots, whereas clinical heterogeneity was examined by assessing the participants, intervention used, study quality and outcome measure. Further sensitivity analysis was performed to assess the heterogeneity and outcome differences between randomized and non-randomized studies. Publication bias was assessed using funnel plots.

Results

The process of literature search and selection of studies for the meta-analysis is shown in **Figure 1**. After the initial screening, 11 studies were further excluded since they did not meet the predefined inclusion criteria for the meta-analysis (**Table 1**). The primary reasons for exclusion were retrospective study design (Lorusso et al., 2008; Chung et al., 2006), retrospective control group (Doldi et al., 2005), lack of control group (Tiboni et al., 2011), timing of intervention either not mentioned (Erlik et al., 2008), remote from IVF cycle (La Sala et al., 1998; Trninic-Pjevic

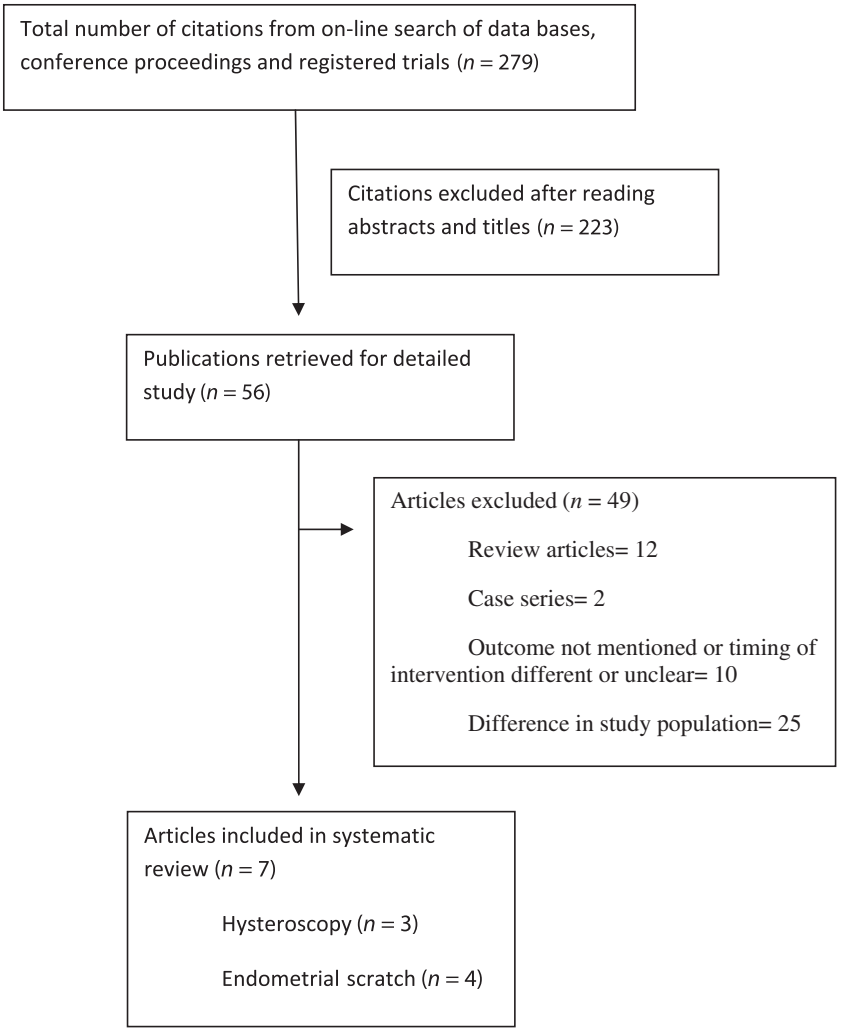


Figure 1 Selection process of the studies for the systematic review.

Table 1 Characteristics of the excluded studies.

Study	Design	Participants	Timing of intervention	Exclusion criteria
La Sala et al. (1998)	NR	Previous 2 failed IVF cycles with good-quality embryos, HSG normal. Intervention: HS and biopsy. Main outcome measure: intrauterine abnormalities	HS and biopsy 1–6 months after last failed IVF	Intervention remote from ovarian stimulation. Outcome not mentioned
Mooney and Milki (2003)	NR	First or failed previous cycle, with ≥3 good-quality embryos, age <40. Intervention: HS (divided as normal cavity and treatment of uterine pathology); control: no HS (remote HS)	Preceding cycle	Different study population
Doldi et al. (2005)	Prospective cases, retrospective control	First IVF–embryo transfer cycle, Infertility of ≥1 year duration, HSG, TVS-normal cavity. Intervention: HS, treatment of abnormality and biopsy	Preceding cycle proliferative phase	Retrospective controls and first IVF–embryo transfer cycle
Chung et al. (2006)	Retrospective	Previous 2 failed cycles despite good-quality embryos, HSG normal. Intervention: HS	Less than a year between HS and fresh IVF cycle	Retrospective
Erlik et al. (2008)	NR	Previous failed 3 IVF cycles, age ≤42, normal HS previously. Intervention: HS and biopsy; control: HS only	Timing not evident	Timing of intervention not mentioned. Control group had hysteroscopy performed
Lorusso et al. (2008)	Retrospective	Mixed cohort, first and previous ≥2 failed cycles, age 18–40, FSH ≤10 mIU/ml. Intervention: HS normal and abnormal	HS 1–6 months preceding ovarian stimulation	Intervention remote from ovarian stimulation
Zhou et al. (2008)	NR	Good responders to hormonal stimulation, endometrium diagnosed irregular by ultrasound. Intervention: endometrial scratch (biopsy)	Ovarian stimulation cycle biopsy during days 5–22	Intervention same cycle of ovarian stimulation
Karimzade et al. (2010)	RCT	First IVF, age <38, FSH <12 mIU/ml, TVS normal. Intervention: biopsy	ovarian stimulation cycle: on the day of oocyte retrieval	Intervention in same cycle as ovarian stimulation
Huang et al. (2011)	NR	Failed 2 IVF cycles with good-quality embryos. Intervention: HS and biopsy	ovarian stimulation cycle HS and biopsy day 2–7	Intervention in the same cycle of ovarian stimulation
Tiboni et al. (2011)	Prospective, no control group	Previous failed IVF cycle	Endometrial biopsy in the preceding cycle	No control group
Trninic-Pjevic et al. (2011)	Not clear	Failed IVF/ICSI cycle where ≥1 good-quality embryos replaced, age <38, TVS normal cavity	HS done 2–6 months prior to ovarian stimulation	Intervention remote from ovarian stimulation

HS = hysteroscopy; HSG = hysterosalpingography; ICSI = intracytoplasmic sperm injection; NR = non-randomized; RCT = randomized controlled trial.

et al., 2011), intervention in the same cycle of ovarian stimulation (Huang et al., 2011; Karimzade et al., 2010; Zhou et al., 2008) and first IVF cycle (Mooney and Milki, 2003).

Seven studies with a total of 2062 participants met the predefined inclusion criteria and were included in the meta-analysis. **Table 2** summarizes the descriptive characteristics of the included studies in this systematic review. Four of these were RCT (Demirol and Gurgan, 2004; Rama Raju et al., 2006; Karimzadeh et al., 2009; Narvekar et al., 2010) and the other three non-randomized trials (Barash et al., 2003; Raziel et al., 2007; Makrakis et al., 2009). In the RCT, two studies compared the effect of hysteroscopy and the other two compared the effect of endometrial biopsy with no intervention. In the non-randomized controlled trials, one compared the effect of hysteroscopy and the two other compared endometrial biopsy with no intervention.

Hysteroscopy studies

Demirol and Gurgan (2004) conducted a RCT on 421 patients aged 24–40 years who had undergone two or more failed IVF cycles in which two or more good-quality embryos were transferred. All the participants presented with primary infertility and had normal uterine cavity and patent tubes at hysterosalpingography (HSG). Participants were randomized using computer-generated numbers (group I, no hysteroscopy, $n = 211$; group II, office hysteroscopy, $n = 210$). A

Table 2 Characteristics of the included studies.

Study	Design	Participants	Timing of intervention	Outcomes	CPR
Hysteroscopy[a]					
Demirol and Gurgan (2004)	RCT	Previous failed ≥2 IVF—embryo transfer in which ≥2 good-quality embryos replaced, age 24—40, HSG normal cavity. Intervention: HS (divided as normal cavity and treatment of uterine pathology); control: no HS	Preceding cycle, early proliferative phase. Office hysteroscopy, 5 mm continuous flow rigid scope, 30° view, saline distension	CPR, complications, abortions	1.52 (1.08—2.15)
Makrakis et al. (2009)	NR	Previous failed IVF attempt, age ≤42, HSG normal cavity. Intervention: HS (divided as normal cavity and treatment of uterine pathology); control: no HS	Preceding cycle, early proliferative phase. Vaginoscopic approach, 5.5 mm continuous flow rigid scope, 30° view, saline distension	CPR, ongoing pregnancy	1.39 (1.13—1.72)
Rama Raju et al. (2006)	RCT	Failed IVF ≥2 cycles, and ≥2 good-quality embryos replaced, age 26—30, HSG normal cavity. Intervention: HS and biopsy (divided as normal cavity and treatment of uterine pathology); control: no HS	Preceding cycle, early proliferative phase. Office hysteroscopy, 3—5 mm continuous flow rigid scope, 30° view, glycine distension	CPR, LBR, miscarriage	1.70 (1.30—2.23)
Endometrial scratch (biopsy)					
Barash et al. (2003)	NR	≥1 failed IVF—embryo transfer cycles, good responders in previous cycle, age 23—45. Intervention: Pipelle biopsy four times; control: no biopsy	Preceding cycle days 8, 12, 21 and 26	CPR, IR, LBR, miscarriage, infection rate	2.20 (1.51—3.20)
Karimzadeh et al. (2009)	RCT	2—6 unsuccessful IVF—embryo transfer, transfer of ≥10 high-grade embryos, age 20—40, poor responders excluded (FSH >10 IU/ml and <4 follicles on day of HCG). Intervention: Pipelle biopsy; control: no biopsy	Preceding cycle luteal phase days 21—26	CPR, IR, complications	3.18 (1.12—9.06)
Narvekar et al. (2010)	RCT	Previous failed cycle with good-quality embryos, age ≤37, HS normal cavity. Intervention: Pipelle biopsy twice; control: no biopsy	Preceding cycle days 7—10 and 24—25	LBR, IR, CPR, infection rates	2.38 (1.07—5.28
Raziel et al. (2007)	NR	≥4 failed embryo transfer of fresh embryos, age ≤40, all investigations for RIF negative, normal cavity, poor responders excluded (FSH >12 IU/l or <4 follicles on the day of HCG). Intervention: Pipelle biopsy twice; control: no biopsy	Preceding cycle days 21 and 26	CPR, IR, ongoing pregnancies, miscarriage, infection rate	2.44 (1.10—5.41)

Values are risk ratio (95% CI).
[a]Subjects who underwent HS treatment for uterine abnormality excluded from meta-analyses.
CPR = clinical pregnancy rate; HCG = human chorionic gonadotrophin; HS = hysteroscopy; IR = implantation rate; LBR = live birth rate; NR = non-randomized; RCT = randomized controlled trial.

total of 154 women of group II had normal hysteroscopic findings while the rest ($n = 56$) underwent hysteroscopic surgery for different identified intrauterine pathologies. The analysis included only women who had normal findings at hysteroscopy and excluded those who underwent operative hysteroscopy. Hysteroscopy was performed in the early proliferative phase of the cycle preceding ovarian stimulation, using a 5 mm continuous flow rigid scope with 30° view and saline distension media. Long down-regulation was commenced from day 21 of the cycle and recombinant FSH was given (225 IU/day) for ovarian stimulation. Day-3 embryo transfer was performed with a maximum of four embryos and luteal support was provided using progesterone pessaries. CPR was significantly higher in the hysteroscopy

group compared with no intervention group (32.5% versus 21.6%, $P < 0.05$; RR 1.52, 95% CI 1.08–2.15), whilst the miscarriage rate was similar in both groups.

Rama Raju et al. (2006) conducted a RCT on 520 patients aged 26–30 years who had two or more failed IVF cycles in which two or more good-quality embryos were transferred and had normal uterine cavity at HSG. Randomization was done using computer-generated random numbers. The intervention group had hysteroscopy performed ($n = 255$) and control group had no intervention ($n = 265$). Those with hysteroscopy and abnormal findings were treated ($n = 95$) and excluded from this meta-analysis. Hysteroscopy was performed in the early proliferative phase of the preceding ovarian stimulation cycle, and at the time of the procedure endometrial sample was also obtained. Hysteroscopy was performed using a 3–5 mm continuous flow rigid scope with 30° view and using glycine distension. Long down-regulation was initiated in the luteal phase of the cycle; recombinant FSH was used for ovarian stimulation. Embryo transfer was performed on day 3 and luteal phase support was given with progesterone pessaries. There was statistically significant difference in the CPR between the hysteroscopy and control group (44.44% versus 26.2%, $P < 0.05$; RR 1.70, 95% CI 1.30–2.23). LBR was also significantly higher in the intervention group (30% versus 16.6% in the control group) and there was no difference in the miscarriage rate.

Makrakis et al. (2009) performed a prospective study on 1475 patients with previous two recurrent implantation failures after IVF despite transfer of at least one good-quality embryo. Patients were ≤42 years and had normal uterine cavity at HSG. All 1475 patients underwent hysteroscopy, 935 had normal findings and 540 had abnormal findings at hysteroscopy, which were treated simultaneously and excluded from this analysis. A matched case–control study was performed for women with normal hysteroscopic findings ($n = 414$) with a control group that had no intervention ($n = 414$). The control group was matched for age, previous implantation failure and normal appearance of the intrauterine cavity. Intervention group had hysteroscopy performed in the early proliferative phase of the preceding ovarian stimulation cycle using a 5.5 mm continuous flow rigid scope with 30° view and saline distension media. Long down-regulation and short protocol were used and ovarian stimulation was achieved using recombinant FSH. After embryo transfer, luteal support was provided with progesterone pessaries and oral oestradiol valerate. CPR was significantly higher in the intervention group compared with the control group (35% versus 25%, $P = 0.002$; RR 1.39, 95% CI 1.13–1.72) and similarly ongoing pregnancy rates were higher in the hysteroscopy group (28.9% versus 21.9%, $P = 0.02$).

Endometrial biopsy (scratch) studies

Karimzadeh et al. (2009) conducted a RCT on 115 patients with history of at least two failed IVF–embryo transfer cycles with transfer of at least 10 high-grade embryos. Participants were aged 20–40 years and poor responders were excluded. Randomization was based on drawing paper from the bag containing equal number of printed-paper for each method. In the intervention group ($n = 58$), endometrial biopsy was obtained in the luteal phase of the preceding ovarian stimulation cycle and the control group had no intervention ($n = 57$). Of the initial 115 patients, nine did not reach oocyte retrieval (four in the biopsy treated and five in the control group) and 13 did not reach embryo transfer (six in the biopsy treated and seven in the control group); therefore, 93 patients were included in the analysis. Long down-regulation was performed in the luteal phase of the cycle and recombinant FSH was used for ovarian stimulation. After embryo transfer, luteal phase support was provided using progesterone pessaries. Baseline characteristics of both groups were similar, whereas CPR was higher in the biopsy as compared with the control group (27.9% versus 8.9%, $P = 0.02$; RR 3.05, 95% CI 1.07–8.66). IR was also higher in the biopsy group (10.9% versus 3.38%, $P = 0.03$) whilst there was no significant difference in the miscarriage rate.

Narvekar et al. (2010) conducted a prospective, open-label RCT on 100 patients who had undergone at least one previous failed IVF–embryo transfer/ICSI cycle, had good response in the previous cycle and were ≤37 years. Randomization was done using computer-generated random numbers and study was not blinded as the patients and the clinicians were aware of the treatment group. All patients had hysteroscopy, but the intervention group ($n = 49$) had endometrial biopsy taken twice, once between days 7–10 and then days 24–25 of the preceding cycle. In the control group ($n = 51$), endometrial biopsy was not obtained. The patients underwent long down-regulation, antagonist or short protocol based on the criteria of age, FSH and antral follicle count. Up to three good-quality embryos were transferred on day 3 and luteal phase was supported by micronized progesterone vaginally. Baseline characteristics and response to hormone stimulation was similar in the two groups. CPR was higher in the intervention group compared with the controls (32.7% versus 13.7%, $P = 0.01$; RR 2.38, 95% CI 1.07–5.28). The LBR and IR were also significantly higher in the biopsy group compared with the control group ($P = 0.04$ for both). No complications were reported in the biopsy group.

Barash et al. (2003) performed a prospective study on a group of 134 patients with one or more previous failed IVF–embryo transfer cycles, age 23–45 years and had good response to hormonal stimulation. Intervention group ($n = 45$) had endometrial biopsy taken four times (days 8, 12, 21, 26) in the preceding cycle of ovarian stimulation. No infections were reported in the biopsy group. The control group ($n = 89$) had no intervention. Long down-regulation protocol was used and two or three embryos were transferred on day 3. Although, 47% of patients underwent double transfer (on days 3 and 5–6 of fertilization), there was no statistically significant difference in the pregnancy outcome based on mode of embryo transfer. Furthermore, the two groups were statistically similar in baseline characteristics and response to hormonal stimulation. CPR was significantly higher in the intervention group compared with the control group (66.7% versus 30.3%, $P = 0.00009$; RR 2.20, 95% CI 1.51–3.20). LBR was also more than 2-fold higher in the biopsy group compared with the controls, as was the IR. Miscarriage rate was similar in the two groups. On the other hand, the multiple pregnancy rate was lower in the biopsy group compared with the controls.

Raziel et al. (2007) prospectively studied 120 couples with high-order implantation failure of ≥4 unsuccessful

embryo transfer of fresh embryos. Participant's age was ≤40 years, with exclusion of poor responders and all other causes of RIF negative. Intervention group ($n = 60$) underwent endometrial biopsy twice on days 21 and 26 of the preceding ovarian stimulation cycle; control group had no intervention ($n = 57$). Long down-regulation protocol was used, ICSI was performed for all subjects and embryo transfer was done on day 3. Participant characteristics were similar in the biopsy and control group. CPR (RR 2.44, 95% CI 1.10—5.41) and IR were higher for the biopsy group, whereas no statistically significant difference was observed for the ongoing pregnancy and miscarriage rates.

Meta-analysis

Primary outcome measure

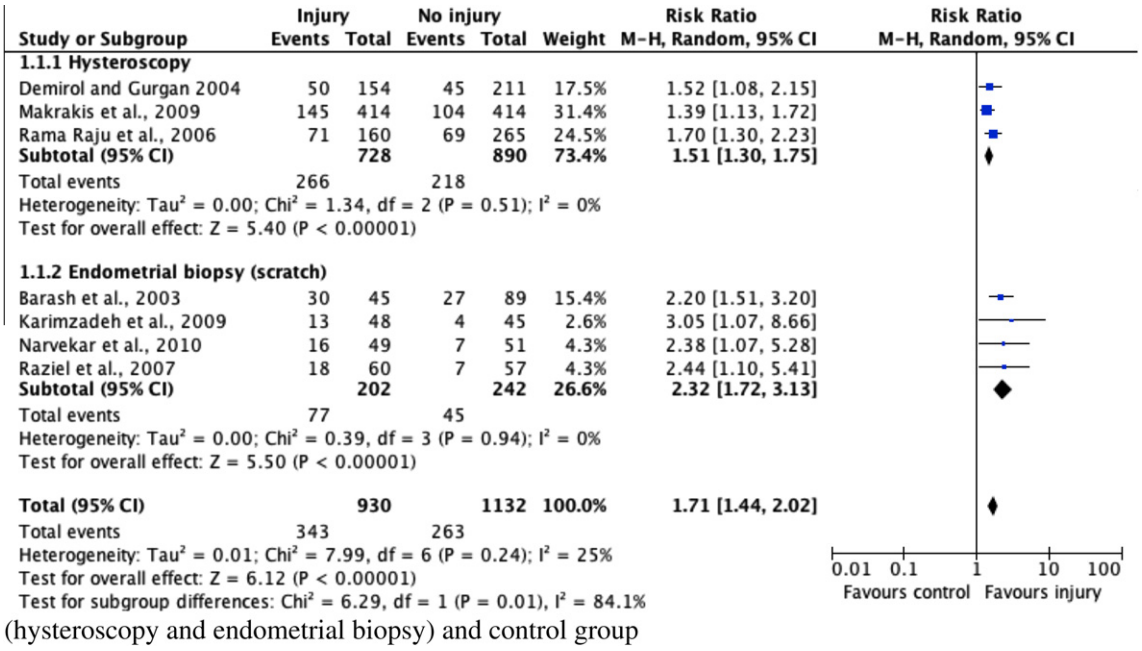
The forest plot for the total effect of hysteroscopy or endometrial biopsy on CPR is presented in **Figure 2**. A total of 728 women underwent hysteroscopy and 202 women underwent endometrial biopsy in the cycle preceding ovarian stimulation for IVF or ICSI as opposed to 1132 women who did not have any intervention before IVF or ICSI. CPR was significantly higher in the hysteroscopy group compared with the control group (36.5% versus 24.5%; RR 1.51, 95% CI 1.30—1.75). Similarly, the CPR was significantly higher in the endometrial biopsy group as opposed to the control group (38.1% versus 18.4%; RR 2.32, 95% CI 1.72—3.13). The CPR for the combined hysteroscopy and endometrial biopsy group was also significantly higher than the non-intervention group (36.8% versus 23.1%; RR 1.71, 95% CI 1.44—2.02). Overall, a small degree of heterogeneity was noted, and it was not statistically significant (chi-squared 8.0, I^2 25%). The test for subgroup differences showed significant difference, $P = 0.01$.

Further sensitivity analyses for the randomized and non-randomized studies showed similar results, and the test for statistical heterogeneity between the two subgroups showed no statistical significance (chi-squared 8.0, I^2 25%) (**Figure 3**). There was no significant difference between the two subgroups, $P = 0.82$. The pooled RR for CPR in the intervention group of RCT was 1.71 (95% CI 1.44—2.02, $P < 0.00001$) and for non-randomized trials was 1.80 (95% CI 1.23—2.64, $P = 0.003$) as compared with the controls.

Secondary outcome measures

Three studies reported the LBR: two RCT and one non-randomized trial (Barash et al., 2003; Narvekar et al., 2010; Rama Raju et al., 2006), with 254 participants in the intervention group and 405 participants in the control group. Meta-analysis of the limited data shows favourable outcome in the endometrial injury group (pooled RR 2.46, 95% CI 1.90—3.18, $P < 0.00001$) (**Figure 4**). Further sensitivity analysis by study design showed LBR higher in the intervention group compared with the control for both randomized and non-randomized trials (RR 2.63, 95% CI 1.94—3.57 versus RR 2.07, 95% CI 1.28—3.34) (**Figure 5**). Four studies on endometrial biopsy reported statistically significant increase in the IR in the intervention group (**Table 3**); however, meta-analysis could not be performed because of difficulty in retrieving the raw data (number of gestational sacs and total number of embryos transferred).

No complications or infection following intervention were reported (**Table 2**). Four studies that reported miscarriages found no difference in the intervention and control groups (RR 0.85, 95% CI 0.59—1.23) (Barash et al., 2003; Demirol and Gurgan, 2004; Rama Raju et al., 2006; Raziel et al., 2007).



(hysteroscopy and endometrial biopsy) and control group

Figure 2 Forest plot for clinical pregnancy rate in the endometrial injury (hysteroscopy and endometrial biopsy) and control groups.

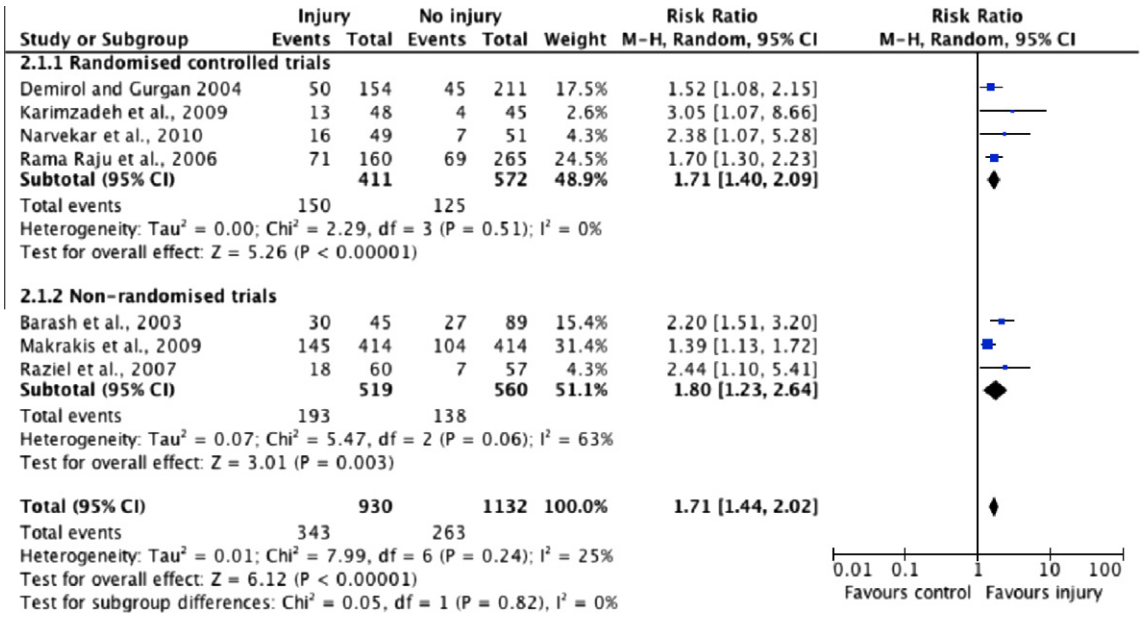


Figure 3 Forest plot for clinical pregnancy rate in the randomized and non-randomized studies for endometrial injury and control groups.

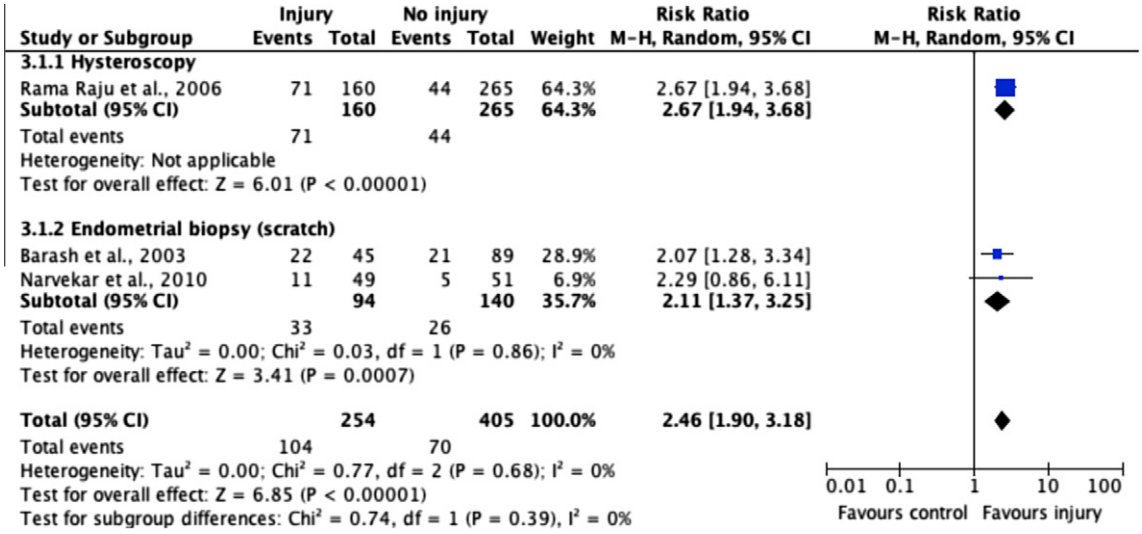


Figure 4 Forest plot for live birth rate in the endometrial injury (hysteroscopy and endometrial biopsy) and control groups.

Discussion

Unexplained RIF is a challenging clinical dilemma and over the last two decades, different treatment options have been studied to improve pregnancy outcomes in this cohort of women. The results of this systematic review and meta-analysis indicate a beneficial effect of inducing local endometrial injury in the preceding ovarian stimulation cycle. The pooled RR for CPR show that local injury is 71% more likely to result in a clinical pregnancy as opposed to no intervention; similarly, the limited meta-analysis for LBR shows 2-fold increase in LBR in the intervention group. Endometrial receptivity is one of the key factors regulating blastocyst implantation and it has been shown that mechanical trauma to the endometrium alters gene expression, enhances secretion of growth factors and makes it more receptive for implantation (Kalma et al., 2009). Interestingly, this effect has been shown to last in the subsequent cycle possibly because the monocytes recruited to the injured sites are long lived and reside in tissues for a long time (Gnainsky et al., 2010).

This systematic review included in the intervention group (endometrial injury) both hysteroscopy and endometrial biopsy/scratch studies because it was believed that even diagnostic hysteroscopy can lead to endometrial trauma/injury, albeit minor compared with pipelle biopsy. Similar to single or multiple biopsies, it is not yet known what degree of injury is required to initiate endometrial receptivity.

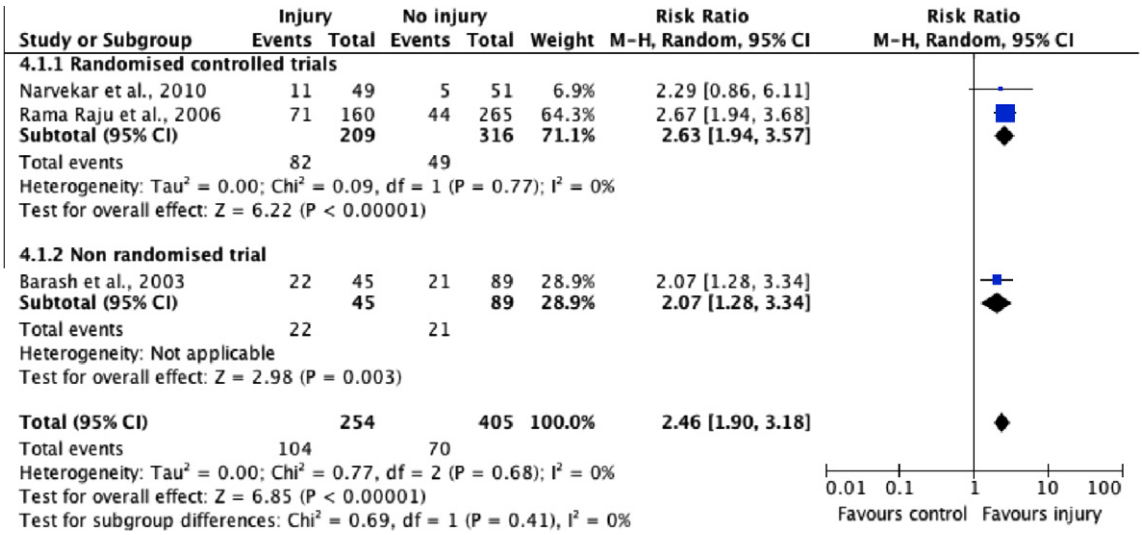


Figure 5 Forest plot for live birth rate in the randomized and non-randomized studies for endometrial injury and control groups.

Table 3 Implantation rates in the intervention and control groups.

Study	*Design*	*Endometrial injury (%)*	*Control (%)*	*P-value*[*]
Barash et al. (2003)	NR	27.7	14.2	0.0001
Karimzadeh et al. (2009)	RCT	10.9	3.38	0.039
Narvekar et al. (2010)	RCT	13.07	7.1	0.04
Raziel et al. (2007)	NR	11.0	4.0	0.02

NR = non-randomized; RCT = randomized controlled trial.
[*]Significance level of <0.05.

With regards to hysteroscopy, a previous systematic review and meta-analysis on outpatient hysteroscopy and subsequent IVF cycle showed improved pregnancy rate following outpatient hysteroscopy (El-Toukhy et al., 2008). In the studies that performed hysteroscopy in the intervention group, participants were subdivided into those with normal and abnormal hysteroscopic findings. Those with abnormalities were treated at the same time. However, Lorusso et al. (2008) showed no significant differences in pregnancy outcome in women with normal and abnormal hysteroscopy (after treatment). Subsequently, Makrakis and Pantos (2010) showed that unsuspected uterine abnormalities are identified in 25—50% of women, and if treated can improve pregnancy outcomes in women with RIF. Intuitively, it is obvious that treatment of intrauterine pathologies is likely to improve implantation, therefore, this review excluded participants who underwent treatment during hysteroscopy and included only those with normal findings to assess the effect of endometrial injury without any bias. In a review on promoting implantation by local injury to the endometrium, Almog et al. (2010) eloquently described that in the studies where hysteroscopy were carried out, this intervention *per se* was the only factor that increased the embryo implantation rate. Similarly, Bozdag et al. (2008) concluded that pregnancy rates improved in women with normal hysteroscopy findings and repeated implantation failure. Although there is evidence regarding improved outcome with hysteroscopy only as an intervention, the mechanism of action has not been identified. It is not obvious whether the hysteroscope itself produces endometrial trauma or whether the distending medium would have an impact. In the present review, none of the studies involving hysteroscopy include flexible hysteroscopes or the use of carbon dioxide.

In some studies that used endometrial biopsy as the intervention, endometrial injury was induced in the same cycle as ovarian stimulation (Huang et al., 2011; Karimzade et al., 2010; Zhou et al., 2008). Zhou et al. (2008) postulated that inducing injury in the stimulated cycle delays endometrial development and improves synchronicity between the endometrium and embryo stage, whereas Karimzade et al. (2010) showed negative impact of endometrial biopsy taken on the day of oocyte retrieval. Huang et al. (2011) even described site-specific injury, on the posterior wall, midline, 10—15 mm from the fundus on days 4—7 of ovarian stimulation. This review refrained from including these studies in the meta-analysis since injury-induced mechanisms for endometrial receptivity are likely to be different in the cycle preceding ovarian stimulation and the stimulation cycle. There is evidence to suggest endometrial gene expression induced with injury varies with the timing of the cycle (Kao et al., 2002; Riesewijk et al., 2003).

Amongst the studies included for the meta-analysis, although the timing of intervention was the 'cycle preceding ovarian stimulation', the phase of the cycle varied. Three studies performed intervention in the early

proliferative phase (Demirol and Gurgan, 2004; Makrakis et al., 2009; Rama Raju et al., 2006); two performed in both the early proliferative and luteal phases (Barash et al., 2003; Narvekar et al., 2010) and the remaining two performed only in the luteal phase (Karimzadeh et al., 2009; Raziel et al., 2007). There is some suggestion that injury induced in the luteal phase is likely to induce more decidualization; however, there is no conclusive evidence to suggest one is better than the other. Moreover, there is insufficient evidence to suggest whether one or multiple biopsies are required to achieve the desired effect on endometrial receptivity. Future RCT should simultaneously look into the molecular and gene expression pathways induced by single or multiple endometrial injuries and the phase of menstrual cycle.

There are indeed some limitations to the current analysis. Firstly, inter-study variation exists because of the inclusion of randomized and non-randomized trials. Two reviewers assessed the quality of the non-randomized trials independently and only prospective trials meeting the predefined criteria have been included. Sensitivity analyses of the RCT and non-randomized trials did not show any difference in the outcome measure, and the tests for statistical heterogeneity and subgroup differences were not significant. The test for subgroup differences between hysteroscopy and endometrial biopsy was statistically significant, which can be due to larger sample size in the hysteroscopy studies compared with endometrial biopsy. Unexplained RIF could have an underlying age related poor response, although only studies with women of age $\leq$40 years have been considered; one prospective trial (Makrakis et al., 2009) that included subjects aged $\leq$42 years was also included because of the good quality of the study and exclusion of poor responders.

The confidence intervals for effect estimates of CPR for some of the individual studies are close to unity (Demirol and Gurgan, 2004; Karimzadeh et al., 2009; Narvekar et al., 2010). This indicates that for these studies statistically significant differences in the effect of the intervention are very small. For the secondary outcomes, the analysis could not provide the effect estimates for IR because of lack of raw data; the four studies, which reported IR, show statistically significant improved outcome in the intervention group. LBR is an important outcome measure in assisted reproductive techniques, and although LBR was reported only by three studies, this analysis performed a limited meta-analysis and a significant benefit was observed in the intervention group.

This meta-analysis provides further evidence that in women with RIF, there is improved pregnancy outcome with local endometrial injury (endometrial scratch/hysteroscopy) performed in the cycle preceding ovarian stimulation. Endometrial injury is associated with local inflammatory response, cascading a release of pro inflammatory cytokines and growth factors like interleukin-6, leukaemia inhibitory factor and tumour necrosis factor α. This further induces decidualization and development of endometrium favourable for embryo implantation. Simultaneously, injury modulates gene expression in the endometrium, with upregulation of pro-implantation protiens such as mucin 1 transmembrane (MUC1), crystallin alpha B, apolipoprotein D (APOD), phospholipase A2 (PLA2) and uroplakin Ib (UPIb) (Kalma et al., 2009). Additionally, Gnainsky et al. (2010) have demonstrated that after endometrial injury there is an increase in macrophages and dendritic cells that play an important role in decidualization and implantation. The monocytes recruited to the injured sites are long lived and reside in the tissues for a long time, thereby supporting the effect of injury induced in the preceding cycle to last in the subsequent cycle.

Overall, with local injury there are changes initiated within the endometrium, the immune system and gene expression, all leading to improved receptivity and a favourable milieu for implantation. It could be that injury in the preceding cycle is more effective as all these events require time and are governed by the hormones. Intervention close to embryo transfer can potentially disturb the endometrium and have a negative effect.

This then raises the clinical question whether there is a role of local endometrial injury in the preceding cycle in all women undergoing IVF or whether it should be limited to women with RIF. The potential benefit of this simple inexpensive procedure could well outweigh the risks of infection and potential of future subfertility. The benefits could be enormous in terms of emotional wellbeing and financial savings. Moreover, what these and related data suggest is that the importance of endometrial receptivity in contributing to IVF pregnancy success is being underestimated. However, there are unanswered questions regarding timing of intervention, phase of cycle when injury should be induced, use of hysteroscopy versus endometrial biopsy, mechanism of action for injury induced with hysteroscopy and benefit of single versus multiple biopsies. There is an urgent need for large, multicentre randomized studies investigating local endometrial injury and pregnancy outcomes in the unexplained RIF and in patients with unexplained subfertility undergoing first IVF cycle. The aforementioned queries need to be addressed and the evidence can be further strengthened by simultaneous molecular and gene expression studies on the endometrium. Until the results of such trials are available, or an expert consortium guideline is proposed, it should not be a blanket policy to induce local endometrial injury for women undergoing first cycle of IVF and perhaps there is a role for this in women with unexplained RIF.

References

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

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

Bozdag, A., Aksan, G., Esinler, I., Yarali, H., 2008. What is the role of office hysteroscopy in women with failed IVF cycles? Reprod. Biomed. Online 17, 410–415.

Chung, Y., Suh, C., Choi, Y., Kim, J., Moon, S., Kim, S., 2006. Effects of endometrial evaluation with office hysteroscopy in IVF–ET patients with repeated failures. Fertil. Steril. 86, S152.

Demirol, A., Gurgan, T., 2004. Effect of treatment of intrauterine pathologies with office hysteroscopy in patients with recurrent IVF failure. Reprod. Biomed. Online 8, 590–594.

Doldi, N., Persico, P., Di Sebastiano, F., Marsiglio, E., De Santis, L., Rabellotti, E., Fusi, F., Brigante, C., Ferrari, A., 2005. Patho-

logic findings in hysteroscopy before in vitro fertilization—embryo transfer (IVF—ET). Gynecol. Endocrinol. 21, 235—237.

Dunn, L., Kelly, W., Critchley, H., 2003. Decidualization of the human endometrial stromal cell: an enigmatic transformation. Reprod. Biomed. Online 7, 151—161.

El-Toukhy, T., Sunkara, K., Coomarasamy, A., Grace, J., Khalaf, Y., 2008. Outpatient hysteroscopy and subsequent IVF cycle outcome: a systematic review and meta-analysis. Reprod. Biomed. Online 16, 712—719.

Erlik, U., Vagman, I., Amit, A., Azem, F., Almog, B., 2008. Endometrial biopsy: a possible vehicle to improve implantation and in vitro fertilization outcome. Fertil. Steril. 90S, S409.

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

Huang, S., Wang, C., Soong, Y., Wang, H., Wang, M., Lin, C., Chang, C., 2011. Site-specific endometrial injury improves implantation and pregnancy in patients with repeated implantation failures. Reprod. Biol. Endocrinol. 9, 140.

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

Kao, L., Tulac, S., Lobo, S., Imani, B., Yang, J., Germeyer, A., Osteen, K., Taylor, R., Lessey, B., Giudice, L., 2002. Global gene profiling in human endometrium during the window of implantation. Endocrine Soc. 143, 2119—2138.

Karimzade, M., Oskouian, H., Ahmadi, S., Oskouian, L., 2010. 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. 281, 499—503.

Karimzadeh, M., Ayazi Rozbahani, M., Tabibnejad, N., 2009. 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. 49, 677—680.

La Sala, G., Montanari, R., Dessanti, L., Cigarini, C., Sartori, F., 1998. The role of diagnostic hysteroscopy and endometrial biopsy in assisted reproductive technologies. Fertil. Steril. 70, 378—380.

Lorusso, F., Ceci, O., Bettocchi, S., Lamanna, G., Constantino, A., Serrati, G., Depalo, R., 2008. Office hysteroscopy in an in vitro fertilization program. Gynecol. Endocrinol. 24, 465—469.

Makrakis, E., Pantos, K., 2010. The outcomes of hysteroscopy in women with implantation failures after in-vitro fertilization: findings and effect on subsequent pregnancy rates. Curr. Opin. Obstet. Gynecol. 22, 339—343.

Makrakis, E., Hassiakos, D., Stathis, D., Vaxevanoglou, T., Orfanoudaki, E., 2009. Hysteroscopy in women with implantation failures after in vitro fertilization: findings and effect on subsequent pregnancy rates. J. Minim. Invasive Gynecol. 16, 181—187.

Margalioth, E., Ben-Chetrit, A., Gal, M., Eldar-Geva, T., 2006. Investigation and treatment of repeated implantation failure following IVF—ET. Hum. Reprod. 21, 3036—3043.

Mirkin, S., Arslan, M., Churikov, D., Corica, A., Diaz, J., Williams, S., Bocca, S., Oehninger, S., 2005. In search of candidate genes critically expressed in the human endometrium during the window of implantation. Hum. Reprod. 20, 2104—2117.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D., 2009. The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. Open Med. 3, e123—e130.

Mooney, S., Milki, A., 2003. Effect of hysteroscopy performed in the cycle preceding controlled ovarian hyperstimulation on the outcome of in vitro fertilization. Fertil. Steril. 79, 637—638.

Narvekar, S., Gupta, N., Shetty, N., Kottur, A., Srinivas, M., Rao, K., 2010. 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. 3, 15—19.

Paria, B., Reese, J., Das, S., Dey, S., 2002. Deciphering the cross-talk of implantation: advances and challenges. Science 296, 2185—2188.

Rama Raju, G., Shashi Kumari, G., Krishna, K., Prakash, G., Madan, K., 2006. Assessment of uterine cavity by hysteroscopy in assisted reproduction programme and its influence on pregnancy outcome. Arch. Gynecol. Obstet. 274, 160—164.

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

Riesewijk, A., Martin, J., van Os, R., Horcajadas, J., Polman, J., Pellicer, A., Mosselman, S., Simon, C., 2003. Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. Mol. Hum. Reprod. 9, 253—264.

Stroup, D., Berlin, J., Morton, S., Olkin, I., David Williamson, G., Rennie, D., Moher, D., Becker, B., Sipe, T., Thacker, S., 2000. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 283, 2008—2012.

Tiboni, G., Giampietro, F., Gabriele, E., Di Donato, V., Impicciatore, G., 2011. Impact of a single endometrial injury on assisted reproductive technology outcome: a preliminary observational study. J. Reprod. Med. 56, 504—506.

Trninic-Pjevic, A., Kopitovic, V., Pop-Trajkovic, S., Bjelica, A., Bujas, I., Tabš, D., Ilić, Đ., Stajić, D., 2011. Effect of hysteroscopic examination on the outcome of in vitro fertilization. Vojnosanit. Pregl. 68, 476—480.

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

Declaration: The authors report no financial or commercial conflicts of interest.

Received 15 April 2012; refereed 20 August 2012; accepted 21 August 2012.