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Endometrial injury for women with previous *in vitro* fertilization failure – does it improve pregnancy rate?

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Abstract

Implantation failure is one of the major factors limiting success of *in vitro* fertilization (IVF) treatment. According to ESHRE 2009 data only 32% of fresh embryo transfers resulted in clinical pregnancies. There are many ideas to improve the treatment outcomes, endometrial injury being one of them. It has been suggested that local endometrial injury, performed either by pipelle biopsy or hysteroscopy, may increase clinical pregnancy rate. However, up to date literature is widely disparate on that subject. There is no conclusion with regard to optimal timing, the number and technique of the procedure. The following paper is the review of the evidence from clinical studies dealing with the effect of endometrial injury on the IVF outcome to guide clinical practice for this challenging problem. PubMed, Embase, the Cochrane Library using Medical Subject Headings and free text terms were searched up to June 2016 without year restriction. Though the majority of trials showed positive impact of endometrial injury on IVF outcome, there is still a lack of strong evidence to support routine local endometrial injury in women prior to IVF treatment.

REVIEW ARTICLE

INTRODUCTION

Assisted reproductive technologies (ART) have become a common treatment for infertility. Despite numerous clinical, embryological and technological improvements in *in vitro* fertilization (IVF) procedure, success rates of these technologies are relatively low (Mikołajczyk *et al.* 2014). It has been indicated that implantation failure is one of the major cause limiting success in IVF treatment. Successful implantation is a compound process that requires a competent embryo interacting with a receptive endometrial lining, under the influence of estrogens and progesterone (Simon *et al.* 2000). The particular mechanism of this interaction still remains unidentified.

According to ESHRE (European Society of Human Reproduction and Embryology) 2009 data only 32% of fresh embryo transfers resulted in clinical pregnancies (Ferraretti *et al.* 2013). There are many ideas to improve the IVF treatment outcomes, such as extended embryo culture, blastocyst selection, assisted hatching and preimplanation genetic screening (PGS) (Hawk *et al.* 1992).

Meanwhile, the vast majority of attempts to improve the IVF implantation rate have focused on PGS, nevertheless successful implantation cannot be assured (Orvieto 2016).Published studies from ESHRE PGD Consortium 2009/10 demonstrated the implantation rates of 22.6% for all women undergoing PGS and 23.9% for women with repeated implantation failure (RIF) (Moutou *et al.* 2014). All the above mentioned methods focused on the embryo. However, several studies have investigated endometrial receptivity as a pivotal factor of embryo implantation. It has been suggested that local endometrial injury in the luteal phase of the cycle preceding embryo transfer may increase the clinical pregnancy rate. The positive effect of this procedure on IVF outcomes was first studied by Barash *et al.* (2003). The mechanical manipulation or local injury to the endometrium can be perfomed by endometrial biopsy or hysteroscopy.

Barash *et al.* and Raziel *et al.* indicated higher implantation rates after endometrial injury in case of RIF, but those were non-randomized studies (Barash *et al.* 2003; Raziel et.al 2007). They were followed by a number of randomized controlled trials resulting in the higher pregnancy rates after endometrial injury (Karimzadeh *et al.* 2009; Narvekar et.al 2010; Gibreel *et al.* 2013). However, more recent researches utilizing a placebo procedure demonstrated no improvement (Baum *et al.* 2012).

In 2015 Lensen *et al.* surveyed 189 fertility clinics and indicated that 83% of them recommended endometrial scratching prior to IVF treatment. Of these, 92% recommended endometrial biopsy to women with recurrent implantation failure (RIF) and 6% recommended it to all women undergoing IVF. 73% of the respondents agreed that this intervention is benficial in women with RIF and 53% reported the lack of benefit among patients undergoing their first IVF cycle (Lensen *et al.* 2016).

Up to date literature is widely disparate on the effect of endometrium injury on IVF outcome. There is no conclusion with regard to optimal timing, the number and techinque of the procedure.

The following paper is the review of the evidence from clinical studies dealing with the effect of endometrial injury on the IVF outcome to guide clinical pratcice for this challenging problem.

THE POTENTIAL MECHANISMS OF POSITIVE EFFECT OF ENDOMETRIAL INJURY

There are three hypothetical mechanisms of beneficial effect of endometrial injury on IVF outcome. Firstly, local injury is thought to induce decidualization of the endometrium, which may increase receptive capacity and improve the implantation of the transferred embryos (Hyodo *et al.* 2011). This hypothesis is based on animal models – guinea pigs (Garris and Garris 2003) and rodents (Finn and Pope 1989), in which scratching uterus provoked the rapid growth of endometrial cells, which are similar to decidual cells of pregnancy.

Secondly, endometrial injury may induce the production of cytokines, growth factors such as leukemia inhibitory growth factor, interleukin-11, heparin-binding endothelial growth factor, macrophages and dendritic cells, all of which are pivotal to embryo implantation (Sherer *et al.* 2001; Basak *et al.* 2002; Siristatidis *et al.* 2014). Gnainsky *et al.* postulated that the concentration of the macrophages/dendritic cells, macrophage inflammatory protein IB (MIP-IB), TNF-α in endometrial samples correlated positively with the improvement of IVF outcome (Gnainsky *et al.* 2010).

Finally, endometrial trauma improves synchronization of endometrium and embryo development by the augmentation of gene expression related to endometrial receptivity. The above include mucin 1 transmembrane, crystallin alpha B, laminin α 4, matrix metalloproteinase-1, apolipoprotein D, uroplakin Ib, phospholipase A2, all of which are supposed to be related to the preparation of the endometrium for embryo implantation through the modification of cellular proliferation, differentation and adhesion (Kalma *et al.* 2009; Dekel *et al.* 2010; Zhou *et al.* 2010). In support of this theory, Junovich *et al.* obtained endometrial samples from oocyte donors during natural and stimulated cycles and measured levels of CD 56+ NK cells. These cells play a crucial role in uterine vascularization and angiogenic factors production (Junovich *et al.* 2011). Junovich *et al.* noticed that ovarian stimulation decreased the concentration of endometrial NK cells, but local injury performed prior to implantation window normalized the levels of CD 56+ NK cells.

THE OPTIMAL TIMING AND FREQUENCY OF ENDOMETRIAL INJURY

Most of the researchers performed single endometrial injury in luteal phase, 7–10 days after the LH surge, of the cycle preceding IVF (Demirol and Gurgan 2004; Rama Raju *et al.* 2006; Karmizadeh *et al.* 2009; Makrakis *et al.* 2009; Yeug *et al.* 2014; Kumbak *et al.* 2014; Singh *et al.* 2015; Elsetohy *et al.* 2015). This particular period is the presumed "window of implantation" with the highest amount of growth factors, cytokines and macrophages/dendritic cells, transcription factors and prostaglandins in the endometrium (Paria *et al.* 2001). Specifically, an increase in interleukin-11 (IL-11) and leukemia inhibitory factor (LIF) expression was noticed in human endometrial cells during the midsecretory phase. The molecular changes during the implantation window include transformation of the fibroblast-like endometrial stromal cells into larger and rounded decidual cells (decidualization), as well as the growth and development of secretory glandules and emergence of large apical protrusions (pinopodes) and microvilli on the luminal epithelium (Paria *et al.*

2001; Dunn *et al.* 2003). Li *et al.* obtained endometrial tissue approximately 2 to 3 weeks before embryo transfer and postulated that this is the sufficient time to achieve favorable effect of endometrial injury on endometrial receptivity (Li *et al.* 2009). It was also shown that endometrial trauma in the luteal phase modulated the expression pattern of genes (HOX cluster such as hoxc10, hoxc11, hoxd10, and hoxd11) involved in implantation, however its effect in the preceding cycle is ambigous, taking into account the fact that during menstruation a lot of endometrial lining is being sched (Kalma *et al.* 2009). However, Gnainsky *et al.* indicated that the positive effect on endometrial receptivity was increased in the cycle that followed endometrial injury (Gnainsky *et al.* 2010). Such long-term effect could be explained by the fact that monocytes enrolled to injured sites are long-living and remain in some tissues for months (McIntire *et al.* 2008). It is also of note that the reduction in endometrial thickness during menstruation mainly affects the spongy layer, leaving most of the stroma and the embedded pro-inflammatory cells unimpaired. Endometrial regeneration occurs from a residuum of the functional rather than from the basal layer.

In contrast to the above mentioned researchers, Karimzade *et al.* performed endometrial injury on the day of oocyte retrieval and showed that it had negative impact on embryo implantation (Karimzade *et al.* 2010). These findings and the previous observations imply that approximately two to three weeks prior to the embryo transfer are needed to achieve favorable effect of endometrial injury on endometrial receptivity.

So far, no conclusion has been drawn with regard to the optimal frequency of endometrial injury. The number of obtained endometrial samples varied from one to four among published trials. However, even Barash *et al.* (2003) who performed four repeated biopsies throughout the menstrual cycle recommended to confine to one endometrial sampling in the secretory phase of the cycle preceding embryo transfer in order to minimize the possible side effects and to make this procedure more acceptable for patients.

WHO IS MOST LIKELY TO BENEFIT FROM ENDOMETRIAL INJURY?

Up to date there is also no agreement with regard to the subgroup of women who might benefit from such treatment. It seems reasonable that this intervention should be profitable for women with recurrent implantation failure, if the hypothesis that uterine receptivity is the cause of implantation failure is true. However, there are insufficient data for women undergoing their first IVF cycle. It is noticable that the majority of the performed trials enrolled younger women with good response to ovarian stimulation. Therefore, this treatment procedure probably will not increase pregnancy rates among poor responders or in the presence of poor embryo quality (Szymusik *et al.* 2015).

ENDOMETRIAL INJURY PERFORMED DURING HYSTEROSCOPY

According to published data, hysteroscopy performed before IVF treatment significantly increases the pregnancy rates in women who had one or more failed IVF cycles (Demirol and Gurgan 2004; Rama Raju *et al.* 2006; El-Toukhy *et al.* 2008; Bosteels *et al.* 2010; Di Spiezio Sardo *et al.* 2016). It is postulated that the immunological effect of endometrial injury is increased by the use of distension medium, which also mechanically removes harmful anti-adhesive glycoprotein molecules from the surface of endometrium (i.e., COX-2, MUC-1 and integrin-αVβ3) (Li *et al.* 2009).

The improvement in the IVF outcome after hysteroscopy was observed by Demirol and Gurgan (2004), who conducted a randomized controlled trial (RCT) in 421 patients aged 24–40 years with primary infertility and with the history of two or more failed IVF-embryo transfer cycles. The hysteroscopy performed in the proliferative phase of the cycle preceding ovarian stimulation, significantly increased clinical pregnancy rate (32.55% in the intervention versus 21.6% in the control group) (Table 1).

In 2006 Rama Raju *et al.* also evaluated the impact of hysteroscopy on IVF outcome in their RCT. They investigated 520 women aged 26–30 years with two or more IVF failures who underwent office hysteroscopy in an early proliferative phase. Statistically significant difference was observed in terms of clinical pregnancy rates between the intervention and control groups (44.44%, versus 26.2%). Live birth rate was also higher in the hysterscopy group (30% versus 16.6%) (Rama Raju *et al.* 2006) (Table 1).

Likewise, Makrakis *et al.* (2009) conducted a prospective study on 1475 patients with a history of two consecutive implantation failures after IVF, who underwent hysteroscopy in the luteal phase of the cycle preceding embryo transfer. The clinical pregnancy rate was significantly higher in study group than in controls (35% versus 25%) and also ongoing pregnancy rate was higher in the intervention group (28.9% versus 21.9%, respectively) (Table 1).

Furthermore, Aghahosseini *et al.* (2012) enrolled and randomized 353 women under the age 38, with normal hysterosalpingogram, who underwent intracytoplasmic sperm injection-embryo transfer (ICSI-ET) after at least two implantation failures. This trial showed that hysteroscopy prior to IVF improved the outcome in recurrent implantation failure patients. Biochemical pregnancy rate was significantly higher in the intervention group – 58.5% versus 34.1% in the control group. The clinical pregnancy rates were 50.7% and 30.3% in hysteroscopy and control groups, respec-

Table 1. Review of trials regarding hysteroscopy vs no intervention prior to IVF treatment

Publication	Design	Participans and inclusion criteria	Intervention	Control	Outcomes intervention group vs. controls
Demirol and Gurgan 2004	RCT	421 women with primary infertility and two or more IVF failures aged 24–40 years	N=210 HSC during proliferative phase of the cycle preceding IVF	N=211 No intervention	CPR* 32.55% vs 21.6%
Rama Raju *et al.* 2006	RCT	520 women with two or more failed IVF/ICSI cycles, mean age 28 years, with normal uterine cavity or unsuspected uterine abnormalities	N=255 Office HSC during proliferative phase of the cycle preceding IVF	N=265 No intervention	CPR* 44.44% vs 26.2%, LBR* 30% vs 16.6%, MR NS
Makrakais *et al.* 2009	NR	1475 women with two implantation failures, aged less than 42 years, normal uterine cavity on HSG	N=414 HSC during proliferative phase of the cycle preceding IVF	N=414 No intervention	CPR* 35% vs 25%, ongoing pregnancy rate* 28.9% vs 21.9%
El-Nashar and Nasr 2011	RCT	124 women scheduled for their first ICSI cycle; mean age 28 years	N=62 HSC with direct biopsy and correction of intrauterine abnormalities encountered. Exact timing of HSC before ICSI not known	N=62 No intervention	CPR* 40.3% vs 24.2%
Aghahosseini *et al.* 2012	RCT	353 women with two or more implantation failures scheduled for ICSI treatment , aged less than 38 years, with unsuspected or no uterine cavity abnormalities. No history of HSC in the last two months	N=142 HSC in the cycle preceding ICSI	N=211 No intervention	Biochemical pregnancy rate* 58.5% vs 34.1%, CPR* 50.7% vs 30.3%, LBR* 35.5% vs 21.1%
Shawki *et al.* 2012	RCT	215 women, aged 22–39 years: 116 scheduled for first ICSI cycle and 99 with one or more failed ICSI cycle. Unsuspected or no uterine abnormalities	N=110 Office hysteroscopy prior to IVF cycles. Exact timing of hysteroscopy prior to controlled ovarian stimulation and embryo transfer not known	N=105 No intervention	CPR* 27.2% vs 38%
Elsetohy *et al.* 2015	RCT	193 women before their first IVF/ICSI treatment; no uterine pathologies besides intramural myomas; mean age 30.5 years	N=97 HSC in the early- or mid- follicular phase. ICSI performed within 3 months from intervention	N=96 No intervention	CPR* 70.1% vs 45.8%
El-Toukhy *et al.* 2016	RCT	702 women younger than 38 years of age, without uterine pathologies visualized during TV US, with the history of two to four unsuccessful IVF cycles	N=350 outpatient HSC within 14 days of menstruation and started the IVF treatment cycle in the following month according to a standard IVF protocol.	N=352 No intervention	CPR 35% vs 33% NS, LBR 29% vs 29% NS

Abbreviations : * – results statistically significant ; NS – no statistically significant; RCT – randomized controlled trial; NR – non randomized trial; N – number of participants; HSC – hysteroscopy IVF – *in vitro* fertilization; ICSI – intracytoplasmic sperm injection**;**
HSG – hysterosalpingogram; IR – implantation rate; CPR – clinical pregnancy rate; LBR – live birth rate; MR – miscarriage rate; vs – versus

tively. The delivery rates were 35.5% in intervention group and 21.1% in controls (Table 1).

Shawki *et al.* (2012) randomized two hundred and forty patients into two groups, 120 patients in group I (ICSI without office hysteroscopy) and 120 patients in group II (had ICSI after office hysteroscopy). At the time of the office hysteroscopy unsuspected uterine abnormalities were found in 33.3% of patients with normal HSG and/or TVS among women in group II. Implantation rate and clinical pregnancy rate were significantly different between group I and group II: 27.2% versus 38%, respectively. An important drawback of this study was that the exact time of intervention was unknown and the group of patients non-homogenous (some were qualified for the first IVF cycle, some with more than one failure) (Table 1).

Contrary to the previously mentioned studies, El-Nashar and Nasr (2011) and Elsetohy *et al.* (2015) evaluated the role of hysteroscopy among women with an unexplained infertility scheduled to start their first ICSI cycle. Elsetohy *et al.* in a randomized controlled trial divided women into two groups. Group I was subjected to hysteroscopic examination within 3 months before ICSI, while group II underwent ICSI without hysteroscopy. Group I showed a significantly higher pregnancy rate (70.1%) versus group II (45.8%). Additionally, there was a significant association between the use of hysteroscopy prior to ICSI and the pregnancy rate (OR 2.77, 95% CI [1.53–5.00]). Moreover, hysteroscopy detected abnormalities in nearly half of the cases with normal ultrasound. El-Nashar and Nasr (2011) examined 124 women with an unexplained infertility. The intervention group – group A (62 women) underwent diagnostic hysteroscopy in which encountered abnormalities were corrected accordingly and directed biopsy performed where necessary; group B (62 women) had no intervention prior to IVF treatment. The clinical pregnancy rate among women in group A was 40.3% compared to 24.2% in group B (Table 1).

Most recently, El-Toukhy *et al.* (2016) in the TROPHY trial investigated whether hysteroscopy improves the live birth rate following IVF treatment in women with recurrent failure of implantation. In this multicentre, randomized controlled trial 350 women under the age of 38, who had normal ultrasound of the uterine cavity and the history of two to four unsuccessful IVF cycles, underwent outpatient hysteroscopy in the month before IVF cycle. They demonstrated that this intervention did not improve the live birth rate, 29% of women in the hysteroscopy group had a livebirth after IVF compared with 29% of women in the control group. However, this trial included patients who had already undergone hysteroscopy before the previous IVF attempts, while other studies enrolled only women who had never undergone hysteroscopy (Table 1).

Although most of the researchers indicated beneficial effect of hysteroscopy on clinical pregnancy rates, the quality of these trials is moderate. The paucity and fragmentation of data, the lack of homogenity of infertile women population who may benefit from this intervention and missing data on exact timing of local endometrial injury make methodological purity relatively low. Moreover, the percentage of papers reporting live birth rates, which are considered the most important end point in ART, was quite low. The high quality RCTs are still necessary to assess the role of hysteroscopy in improving reproductive outcomes.

ENDOMETRIAL INJURY PERFORMED BY PIPELLE BIOPSY

One of the first researchers who suggested the favorable effect of endometrial scratching was Barash. In the prospective case – control non – randomized study the endometrial biopsy performed on days 8, 12, 21 and 26 of the cycle prior to IVF treatment, doubled the chance for a take-home baby (Barash *et al.* 2003). Subsequently, various other publications indicated positive effect of this procedure. Raziel *et al.* (2007) in a non – randomized controlled trial included 60 women, who underwent endometrial injury on days 14 and 19 of the cycle preceding IVF treatment. The clinical pregnancy rate was 30% in the intervention group versus 12% in the control group. Moreover, the ongoing pregnancy rate per ET was higher in the biopsy group than in the controls (22% versus 8% respectively) (Table 2).

Afterwards 2 randomized controlled trials (RCTs) (Karimzadeh *et al.* 2009; Narvekar *et al.* 2010) and 3 meta-analyses (El-Tokhy *et al.* 2012; Potdar *et al.* 2012; Nastri *et al.* 2015) concluded that endometrial biopsy prior to IVF procedure increased clinical pregnancy rates and live birth rates. It is of importance that those meta – analyses included mostly non-randomized studies and only a limited number of the available randomized trials were taken into account (Nastri *et al.* 2015). Karmizadeh *et al.* (2009) performed pipelle biopsy once in the luteal phase using the biopsy catheter – Pipelle de Cornier. The implantation rate was determined as 10.9% in the biopsy group compared to 3.38% in the controls. The clinical pregnancy rate was significantly higher in the case group than in controls (27.1% and 8.9%, respectively) (Table 2). Narvekar *et al.* (2010) performed pipelle biopsy twice. The patients in the intervention group underwent endometrial injury with a biopsy catheter, first on the day of hysteroscopy (between 7^{th}–10^{th} day of the cycle preceding embryo transfer) and once again on day 24^{th} –25^{th} of the same cycle. Such intervention resulted in 13.07% versus 7.1% implantation rate, 32.7% versus 13.7% clinical pregnancy rate and 22.4% versus 9.8% live birth rate per ET compared to control group (Table 2). It is of note that both Karmizadeh *et al.* and Narvekar *et al.* did not have a well-defined primary outcome and the number of studied groups was small. Besides, Karimzadeh *et al.* did not mention allocation concealment of studied participants nor the live birth rate.

Table 2. Review of trials regarding endometrial biopsy vs no intervention prior to IVF treatment.

Study	Design	Participans and inclusion criteria	Intervention group	Control group	Outcomes
Barash *et al.* 2003	NR	134 women with more than one failed IVF – embryo transfer, normal responders, aged 23 – 45 years	N=45 Pipelle biopsy in the preceding cycle on days 8,12, 21 and 26	N=89 No biopsy	CPR* 66.7% vs 30.3%, IR* 27.7% vs 14.2%, LBR* 48.9% vs 22.5%, MR* 22.2% vs. 22%
Raziel *et al.* 2007	NR	117 women with at least four implantation failures, age less than 40 years, normal responders, normal uterine cavity	N=60 Pipelle biopsy in the preceding cycle between days 21–26	N=57 No biopsy	CPR* 30% vs 12%, IR* 11% vs 4%, ongoing pregnancies* 22% vs 8% MR* 28% vs 28%,
Karimzadeh *et al.* 2009	RCT	93 women aged between 20 and 40 years with at least two unsuccessful IVF transfers of high grade embryo, normal responders,	N=48 Pipelle biopsy in the preceding cycle between days 21–26	N=45 No biopsy	CPR* 27.1% vs 8.9%, IR* 10.9% vs 3.38%,
Narvekar *et al.* 2010	RCT	100 women with at least one failed IVF cycle with high grade embryos, age less than 37 years and normal uterine cavity visulized during HSG	N=49 Pipelle biopsy in the preceding cycle between days 7–10 and 24–25	N=51 No biopsy	IR* 13.07% vs 7.1%, CPR* 32.7% vs 13.7%, LBR* 22.4% vs 9.8%
Karimzade *et al.* 2010	RCT	156 women in their first IVF/ICSI, age less than 38 years with good ovarian reserve and without abnormalities visualized during USG TV	N=77 two small endometrial samples were obtained with a Novak curette on the day of oocyte retrieval	N=79 No biopsy	IR* 12.3% vs 32.9%, CPR* 9.6% vs 29.1%,
Baum *et al.* 2012	RCT	36 women with RIF (three or more unsuccessful cycles of IVF-ET) undergoing IVF, aged between 18 and 41 years with good ovarian response in previous cycles	N=18 Endometrial Pipelle biopsy performed between days 9–12 and 21–24 of the spontaneous menstrual cycle preceding IVF	N=18 No biopsy	IR 2.08% vs 11.11% NS CPR 0% vs 31.25% NS, LBR 0% vs 25% NS
Gibreel *et al.* 2013	RCT	105 couples with an unexplained infertility, women aged between 20 and 39 years, regular, ovulatory cycles and bilateral tubal patency, no data regarding previous IVF procedures	N=54 women who underwent endometrial scratching in the luteal phase of a spontaneous menstrual cycle	N=51 No biopsy	CPR 25.9% vs 9.8%, MR* 12.5% and 16.5%
Kumbak *et al.* 2014	NR	128 normal responders, age less than 40 years, with normal uterine cavity visulized during HSC within the previous 6 months	N=70 Office hysteroscopy and concurrent endometrial biopsy were performed in the luteal phase, on the day of GnRH agonist initiation preceding ET cycle	N=58 women GnRH agonist was initiated without any interven-tion.	IR* 38% vs 25%, CPR* 67% vs 45% Ongoing pregnancy rates 54% vs 38% NS
Yeung *et al.* 2014	RCT	300 unselected subfertile women scheduled for IVF/ICSI treatment with normal uterine cavity visualized during saline infusion sonogram (SIS) or HSC.	N=150 Endometrial Pipelle biopsy performed in mid-luteal phase preceding IVF cycle	N=150 No biopsy	IR 32.8% vs 32% NS, CPR 34% vs 38% NS, Ongoing pregnancies 26.7% vs 38% NS MR 30.3% vs 16.6% NS
Singh *et al.* 2015	RCT	60 women with a history of more than one previous failed IVF-ET cycles, age less than 35 years, good ovarian reserve (antral follicle count [AFC] >8, anti-Mullerian hormone [AMH] 2–6 ng/ml, no uterine procedures within the last 3 months	N=30 Single endometrial scratching between days 14–21 of menstrual cycle prior to embryo transfer	N=30 No biopsy	IR* 19.4% vs 8.1%, CPR 16.7% vs 0.0% NS , LBR 10.3% vs. vs 3.3% NS, MR 6.7% vs. 3.3% NS,

Abbreviations : * – results statistically significant ; NS – no statistically significant; RCT – randomized controlled trial; NR – non randomized trial; N – number of participans; HSC – hysteroscopy IVF – *in vitro* fertilization; ICSI – intracytoplasmic sperm injection**;** HSG – hysterosalpingogram; IR – implantation rate; CPR – clinical pregnancy rate; LBR – live birth rate; MR – miscarriage rate; vs – versus

Moreover, Kumbak *et al.* (2014) in a prospective, non-randomized, controlled study showed that office hysteroscopy and concurrent endometrial biopsy performed in the luteal phase on day 21 of the cycle preceding embryo transfer significantly improved the implantation rate (38% versus 25%) and IVF outcome (pregnancy rate 67% versus 45%). However, no significant difference was noticed with regard to ongoing pregnancy rate (Table 2).

Furthermore, Baum *et al.* (2012) who performed pipelle biopsy twice on day 9–12 and 21–24 of the menstrual cycle and Yeung *et al.* (2014) who aspirated endometrium 7 days after the LH surge in ovulatory patients and on 21st day of the cycle in anovulatory patients demonstrated no significant improvement in ongoing pregnancy rates. Both of the studies were RCTs (Table 2). Moreover, some investigators showed a negative impact of endometrial scratching on implantation and IVF outcome. Karimzade *et al.* (2010) who performed endometrial biopsy on the day of oocyte retrival pointed out the adverse effect of this intervention – clinical pregnancy rate dropped to 12.3% vs 32.9% in controls, ongoing pragnancy rate to 9.6% vs 29.1%, respectively (Table 2).

Gibreel *et al.* (2013) assessed whether endometrial scratching improved live birth rate in women with an unexplained infertility undergoing fresh IVF cycle. In an RCT 105 patients were allocated into two groups. They performed endometrial biopsy procedure twice in the luteal phase of the cycle. They demonstrated that endometrial scratching might improve the outcome of IVF treatment (clinical pregnancy rate was 25.9% in intervention group and 9.8% in control group). However, the studied group was not homogenous, there was no data on previous IVF treatment procedures (Table 2).

A recent study by Singh *et al.* (2015), including 60 women with a history of more than one previous failed IVF-ET cycles who underwent endometrial scratching once between days 14 and 21 of the cycle prior to ET, demonstrated that the implantation rate was significantly higher in the study group (19.4%) than in the control group (8.1%). Though, there were no significant differences in the ongoing pregnancy and live birth rates between the groups (Table 2).

CONCLUSION

Currently, there is lack of strong evidence to support routine endometrial injury in women prior to IVF treatment. That is because there are still many unanswered questions, such as the technique, number, timing of the procedure and the duration of a gap between endometrial biopsy and embryo transfer that need to gain consensus. Definitely a well-designed randomized controlled trial demonstrating the impact of endometrial injury on live birth outcomes is required. It should be performed on the homogenous group of selected patients, which is never easy in human IVF. The ongoing multi-centre trial „Pipelle for Pregnancy (PIP): study protocols for three randomized controlled trials" (Lensen *et al.* 2016), which is designed to test whether endometrial injury increases the probability of live birth rate in women, seems to be a promising solution for all the above mentioned dilemmas.

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