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Impact of luteal phase hysteroscopy and concurrent endometrial biopsy on subsequent IVF cycle outcome

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Abstract
Purpose Endometrial biopsy preceding implantation in in vitro fertilization (IVF) treatment causes a type of injury which facilitates implantation. Pre-treatment hysteroscopic evaluation of uterine cavity also raises the success in IVF. This study investigates whether office hysteroscopy and concurrent endometrial biopsy performed in the luteal phase, on the day of GnRH agonist initiation for long protocol, improves subsequent IVF outcome.
Methods A prospective, nonrandomized, controlled study of 128 normoresponder women was performed: In 70 women (study group), office hysteroscopy and concurrent endometrial biopsy were performed on the day of GnRH agonist initiation preceding ET cycle and in 58 women (control group), GnRH agonist was initiated without any intervention. However, uterine cavity was shown to be normal with hysteroscopy within the previous 6 months in those women. Implantation and pregnancy rates were compared between the groups.
Results Intrauterine pathologies were observed in 28 % of women in the study group. Implantation rate (38 vs. 25 %; $p = 0.04$) and pregnancy rate per ET (67 vs. 45 %; $p = 0.01$) were found to be significantly higher in the study group compared to the control group.
Conclusion Office hysteroscopy and concurrent endometrial biopsy performed in the luteal phase, on the day of GnRH agonist initiation for long protocol, provide direct evaluation of the uterine cavity immediately before ET cycle and also significantly improve the implantation and IVF outcome.

Keywords Endometrium · Endometrial injury · Hysteroscopy · Implantation · In vitro fertilization

Introduction

Implantation in in vitro fertilization (IVF) treatment has been maximized up to 70 % with blastocyst transfer in good responder patients [1]. However, the other aspect of implantation which is endometrial receptivity is still one of the most vigorously investigated topics in assisted reproduction. Recently, some authors have suggested a favorable effect of endometrial scratching which is a type of endometrial stimulation on the success rate especially in women with previous implantation failure [2–6]. However, patients included, timing, number and the technique of endometrial infliction(s) are variable in the studies [5].

The prevalence of intrauterine pathologies not suspected on TVS but found at hysteroscopy (HS) has been reported to be up to 45 % [7–9]. Those unsuspected intrauterine abnormalities are considered to decrease the probability of pregnancy in IVF, therefore, it is advised to diagnose and treat those pathologies prior to IVF in order to maximize the treatment success although not evidence-based yet [10, 11]. For the evaluation of the uterine cavity, office HS is

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becoming a routine procedure especially in patients undergoing repeated IVF cycles but cannot conceive [10, 12–14]. It has advantages like being an outpatient procedure, having no need for anesthesia or an operating room, enabling direct visualization, diagnosing and treating the pathology simultaneously [15].

In recent studies, HS performed in the cycle preceding IVF was reported to improve pregnancy rate [16, 17]. Furthermore, it has been suggested to time HS as close to the IVF cycle as possible and, to consider repeating HS in patients with multiple failed IVF treatments [18].

The aim of this study is to investigate the impact on IVF outcome of office HS and concurrent endometrial biopsy performed in the luteal phase, on the day of GnRH agonist initiation, in women undergoing treatment with long protocol.

Materials and methods

Between April 2011 and April 2012, we prospectively recruited 128 consecutive normo-ovulatory women who were planned to undergo IVF treatment with long luteal agonist protocol at our university hospital IVF unit. Women with age >40 years, cycles other than 28–32 days, polycystic ovary syndrome, poor response in previous IVF treatments (<5 oocytes retrieved), uterine anomaly, stage III–IV endometriosis and, sonographically detected hydrosalpinx were excluded. Approval for this prospective cohort study was obtained from the institutional review board. All the patients gave informed consent for the treatment received.

In all women, GnRH agonist (Lucrin®, Abbott) was initiated at 0.5 mg daily dose on the 21st day of the cycle. On the third day of the subsequent cycle, an ultrasound was performed and serum estradiol was measured. In case serum estradiol <50 pg/ml and endometrial thickness <5 mm with no follicles >10 mm in diameter, gonadotropins were commenced at 150–300 IU/day dose individualized according to woman age, BMI and ovarian reserve. When the leading follicle reached 20 mm, recombinant HCG (Ovitrelle®, Serono) 250 mg was administered and oocyte pick-up was scheduled 36 h afterwards. ICSI was performed with fresh ejaculated sperm and embryo transfer 3–5 days after oocyte pick-up. Luteal phase support was given with 50 mg i.m. progesteron daily until the pregnancy test. Serum beta HCG was measured 12 days following embryo transfer (ET) to reveal pregnancy.

Implantation rate was calculated as the ratio of the number of gestational sacs to total number of embryos transferred. Miscarriage was defined as a pregnancy which ended within the first 12 gestational weeks. Ongoing pregnancy or live birth rate was expressed as the ratio of the number of ongoing pregnancies or births to the total number of patients who underwent ET.

In the study group, comprising of 70 patients, office HS was performed on the 21st day of the cycle preceding ET cycle using a rigid, 30°, 4-mm hysteroscope (Karl Storz Endoscopy, Tuttlingen, Germany) without anesthesia. In cases of inability to pass through the internal ostium, cervical dilatation until the passage of hysteroscope was performed under mild sedation. Interventions such as polyp removal or subseptum resection were performed using a scissor or a grasping forceps and an endometrial sample was obtained using a biopsy catheter (Pipelle, Gynetics Medical Products, Belgium) and sent for histopathological examination at the end of HS procedure. In patients with chronic endometritis, doxycycline 100 mg bid for 10–14 days was administered before starting ovarian stimulation. In the control group, comprised of 58 patients, GnRH agonist was initiated on the 21st day of the cycle without performing HS or endometrial biopsy, only evaluation with TVS was done (Famio 8, Toshiba, Japan). Albeit, patients in the control group were already evaluated with office HS within the previous 6 months.

Results were expressed in terms of mean.values and standard deviations. The normality of data was checked by Kolmogorov–Smirnov test. Normally distributed continuous variables were analyzed by Student's t test. Categorical variables were compared with the Chi-square test and expressed as numbers or percentages. Results were considered statistically significant at $p < 0.05$. All statistical analyses were conducted by using SPSS 12.0 (SPSS Inc., Chicago, IL, USA).

Results

No difference was found between the two groups as regards patient characteristics (Table 1). Concerning the cycle characteristics, the amount of gonadotropins required was higher (2,965 vs. 2,467 IU; $p = 0.02$), total number of retrieved oocytes was lower (14 vs. 18; $p = 0.01$) and, the total number of mature oocytes obtained was also lower (11 vs. 14; $p = 0.006$) in the study group compared to the control group (Table 1).

Regarding the outcome parameters, fertilization rate was higher (82 vs. 73 %; $p = 0.009$) in the study group compared to the control group (Fig. 1). Although the number of transferred grade I embryos was similar, implantation rate (38 vs. 25 %; $p = 0.04$) and the pregnancy rate per ET (67 vs. 45 %; $p = 0.01$) were found to be significantly higher in the study group compared to the control group (Fig. 1). However, no significant difference was noticed between the two groups with regard to miscarriage rate or ongoing pregnancy/live birth rate (Fig. 1).

Table 1 Patient and cycle characteristics

Characteristics	HS and endometrial sampling group ($n = 70$)	Control group ($n = 58$)	p value
Age (years)	29.5 ± 3.6	29.4 ± 3.9	NS
BMI (kg/m^2)	24.4 ± 4.5	25.9 ± 4.8	NS
Day 3 FSH (IU/l)	6.3 ± 2.5	5.7 ± 2.4	NS
Infertility duration (years)	6.0 ± 3.5	6.1 ± 3.6	NS
Infertility etiology, *n* (%)			NS
Unexplained infertility, *n* (%)	34 (48)	28 (48)	
Male factor, *n* (%)	25 (36)	24 (42)	
Endometriosis, *n* (%)	3 (4.5)	3 (5)	
Tubal factor, *n* (%)	8 (11.5)	3 (5)	
Total gonadotropins used (IU)	*2,965 ± 1,329*	*2,467 ± 963*	*0.02*
Serum E2 on HCG day (pg/ml)	2,720 ± 1,222	3,077 ± 1,261	NS
Endometrium on HCG day (mm)	10.8 ± 2.1	11.1 ± 2.2	NS
Total oocytes retrieved (*n*)	*14.2 ± 6.3*	*17.6 ± 8.7*	*0.01*
Total mature oocytes retrieved (*n*)	*10.7 ± 5.3*	*13.7 ± 7.0*	*0.006*
Transferred grade I embryos (*n*)	1.5 ± 0.7	1.6 ± 0.9	NS
ET on day 5 (blastocyst stage) (%)	60	43	NS

Chi-square test, Student's *t* test

Italic values represent significantly different in the study group compared to the control group

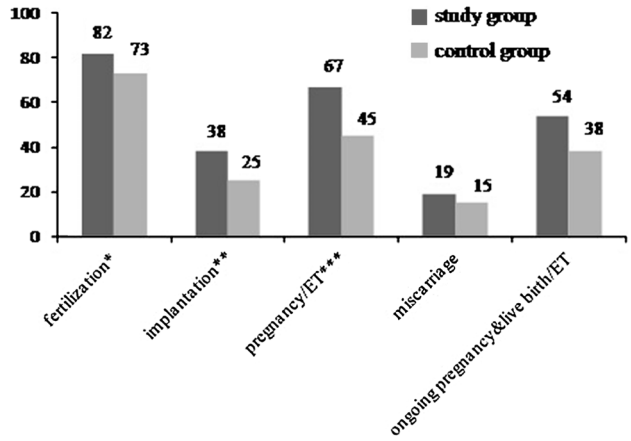

Fig. 1 Outcome parameters in patients who underwent office HS and concurrent endometrial biopsy on the day of GnRH agonist initiation (study group) and in those who had no intervention (control group). The *numbers* are percentages. Fertilization rate, implantation rate and pregnancy rate per embryo transfer were significantly different between the groups. Miscarriage rate and ongoing pregnancy and live birth rate per embryo transfer were similar. *$p = 0.009$, **$p = 0.04$, ***$p = 0.01$

Intrauterine pathologies were found with HS in 28 % of women in the study group. The pathologies detected were chronic endometritis (11 %), endometrial polyp (8 %), subseptum (6 %) and endometrial hyperplasia (3 %). No complications occurred and patients were discharged 2 h after the procedure. In six patients (9 %), hysteroscope could not be introduced easily through the internal cervical ostium. In four of them, cervical dilatation was performed and in the other two who could not tolerate cervical dilatation, misoprostol was given at night and the procedure was performed on the next day without any difficulty.

Discussion

Advances in assisted reproductive treatments provided achievement of good quality embryos, however, it is still difficult to attain a receptive endometrium during the window of implantation. It is suggested that inadequate endometrial receptivity is responsible for approximately two-thirds of implantation failures [19]. Recently a favorable effect of endometrial injury induced by a biopsy catheter on the implantation, clinical pregnancy and live birth rates has been reported [5]. More recently, office HS has been recommended as a routine investigation before IVF even in patients with normal hysterosalpingography and/or transvaginal sonography [11]. The present study demonstrates that office HS and concurrent endometrial biopsy performed in the luteal phase, on the day of GnRH agonist initiation, improves implantation in the subsequent cycle in women undergoing IVF and also provides evaluation of the uterine cavity immediately before ET cycle.

Endometrial biopsies taken at various cycle days have been shown to exert a favorable effect on implantation and pregnancy outcome [2–4, 20]. The explanation for this beneficial effect is not yet fully clear. Gnainsky et al. [21] have suggested that biopsy-induced local injury elicits an

inflammatory reaction which facilitates implantation. Natural killer cells, macrophages and dendritic cells infiltrate the injured site and secrete increased amounts of cytokines, growth factors and chemokines resulting in successful implantation [21, 22]. Endometrial biopsy-induced injury has also been hypothesized to increase endometrial receptivity by modulating the expression of a variety of genes [23].

Timing, number and the technique of endometrial injury are variable in the literature (Table 2) [5, 24]. In the previous studies, endometrial sampling with Pipelle biopsy catheter was confined to patients with one or more previous failed IVF cycles and performed 1–4 times either in the follicular or the luteal phase in the cycle preceding IVF (Table 2) [2, 3, 6, 20]. Although multiple biopsies were performed in some studies, it was suggested that performing biopsy only during the secretory phase would be sufficient both to decrease patient discomfort and also because injury-induced decidualization was shown to be most effective under the influence of progesterone [2, 25]. In the present study, only once, on the day of agonist initiation in the luteal phase, an endometrial injury was done and most of the patients were in their first IVF treatment. Only 19 patients (27 %) in the study group and 14 patients (24 %) in the control group had one or two previous failed cycles. We performed subgroup analysis in those patients and found similar implantation rates (33 and 15 %, respectively). Although the value is higher in the study group, it did not reach statistical significance. Limited patient number might be the reason.

Interestingly nearly all of the embryo transfers were day 2–3 transfers in the previous studies; however, blastocyst stage transfer improves implantation and pregnancy rate in women with recurrent implantation failure [2–4, 6, 26]. Day 2–3 embryo transfer is very unlikely in excluding embryonic effect on implantation. In our study most of the embryo transfers were of blastocyst stage.

Evaluation of the uterine cavity is one of the basic steps in infertility work-up. Intracavitary abnormalities might cause both implantation failure and also spontaneous abortion [11]. In a study by Feghali et al. [27] outpatient HS was performed in 145 women before the first IVF cycle and intrauterine pathologies were observed in 45 % of hysteroscopies. Systematic use of HS before IVF is a widely accepted practice which is supposed to improve treatment outcome but still lacks scientific evidence [16]. Especially in patients with previous implantation failure, uterine cavity should be evaluated with HS which has been reported to improve pregnancy rate [7, 10, 16, 28]. However, the value of the performance of HS prior to first IVF cycle remains to be established [8, 29]. In a previous study of 300 women evaluated with HS before proceeding to their first IVF cycle, pregnancy rate in women who underwent pre-IVF HS was found to be significantly higher compared to those who did not [30]. In the current study, patients in both the study and the control groups were

Table 2 Studies in the literature about endometrial injury and its effect on IVF outcome

References	EI no.	EI timing	EI technique	Impact on IVF	Patients included
Barash et al. [2]	4×	Days 8, 12, 21 and 26 of the cycle before IVF-ET	Pipelle biopsy catheter	IR, CPR, LBR ↑	134 good responder women, with ≥1 IF, 45 biopsy-treated vs. 89 no intervention
Raziel et al. [3]	2×	Days 21 and 26 of the cycle before IVF-ET	Pipelle biopsy catheter	IR and OPR ↑	117 women, with ≥4 IF, 60 biopsy-treated vs. 57 no intervention
Zhou et al. [4]	1×	Days 5–22 of COH cycle	No. 5 biopsy catheter	IR, CPR, LBR ↑	121 good responder women, whose endometria were found to have irregular echopattern on TVS, 60 biopsy-treated vs. 61 no intervention
Narvekar et al. [6]	2×	Days 7–10 and 24–25 in the cycle preceding the ET cycle	Pipelle biopsy catheter	IR, CPR, LBR ↑	100 women, with ≥1 IF, 49 biopsy-treated vs. 51 no intervention
Karimzadeh et al. [20]	1×	Luteal phase of previous cycle	Biopsy catheter	IR, CPR ↑	115 women with ≥2 IF
Karimzade et al. [24]	1×	Day of OPU	Novak curette	IR, CPR, OPR ↓	156 women in their first IVF cycle, 77 biopsy-treated vs. 79 no intervention

EI endometrial injury, *IR* implantation rate, *CPR* clinical pregnancy rate, *LBR* live birth rate, *OPR* ongoing pregnancy rate, *TVS* transvaginal sonography, *IF* implantation failure, *OPU* oocyte pick-up

evaluated with HS; in the study group women underwent HS immediately before IVF cycle and in the control group, HS was performed previously within 6 months of IVF. We found significantly better outcome in the study group. This probably has two reasons: first we might have made an injury to the endometrium increasing implantation, and the second reason is that more recent HS provided the detection and the treatment of any intrauterine abnormality immediately before ET cycle. However, hysteroscopic findings were similar in the two groups. Similarly some others also proposed that HS per se increased the implantation and the pregnancy rates regardless of the findings [10, 28].

Considering the psychological distress an IVF patient experiences with every failed treatment cycle, it is urgent to reveal the value of HS as a routine procedure for the assessment of uterine cavity prior to IVF [8]. Recently HS has been suggested to be performed before IVF even in women with normal hysterosalpingography or TVS [11].

In order to evaluate the uterine cavity thoroughly, some authors performed HS in the follicular phase and even under oral contraceptive use [8, 9]. In this study, we did not have any difficulty with the visualization of the cavity or either ostia. However, in six cases it was difficult to enter the cavity in the midluteal phase. In four of them, cervix was dilated under mild sedation, in the remaining two patients who could not tolerate cervical dilatation, misoprostol was given intravaginally at night and the procedure was performed easily on the next day.

In conclusion, the results of the current study propose that performance of office HS and concurrent endometrial biopsy on the day of GnRH agonist initiation in a long luteal controlled ovarian hyperstimulation cycle both provides intrauterine evaluation immediately before ET cycle and also a way to make an endometrial injury which significantly improves the outcome.

Conflict of interest The authors declared no conflicts of interests. The authors state that they have full control of all data and agree to allow the Journal to review the data if requested.

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