“Chromohysteroscopy” for evaluation of endometrium in recurrent in vitro fertilization failure

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Received: 28 November 2007 / Accepted: 20 December 2007 / Published online: 29 January 2008
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Abstract

Purpose To assess the efficacy of chromohysteroscopy in detecting endometrial pathologies in recurrent in vitro fertilization (IVF) failure.

Materials and methods Sixty-four patients in whom conventional hysteroscopy did not show any apparent endometrial pathology were included. Five milliliter of 1% methylene blue dye was introduced through the hysteroscopic inlet. Biopsies were obtained both from dark stained and light stained areas.

Results The study group was grouped according to the staining characteristics. Group I included 22 patients in whom focal dark staining was observed. Group II included 41 patients in whom diffuse light blue staining without dark areas was observed. There was no significant difference between two groups in age, smoking, body mass index, number of IVF failure and time to hysteroscopy after the last failure. But, there was a statistically significant difference in the incidence of endometritis between two groups ($p=0.007$). The power of dark staining for detection of endometritis was calculated as follows: sensitivity 69.2%, specificity 74%, positive predictive value 40.9% and negative predictive value 90.2%.

Capsule Chromohysteroscopy improves the efficacy of hysteroscopy in recurrent IVF failure.

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Conclusion Chromohysteroscopy improves the efficacy of hysteroscopy in recurrent IVF failure. Observation of diffuse light blue staining without dark areas strongly suggests a normal endometrium free of endometritis.

Keywords IVF failure · Hysteroscopy · Chromohysteroscopy · Endometritis

Introduction

Uterine integrity and endometrial receptivity are among major factors affecting the success of assisted reproduction. When two cycles of in vitro fertilization (IVF) fail despite good-quality embryo transfer (ET), uterus/endometrium is the first factor to be re-evaluated [1]. The evaluation of uterine factor can be accomplished using ultrasonography, hysterosalpingography (HSG) and hysteroscopy. Hysteroscopy has the advantage in its ability to establish a visual diagnosis. When there is a macroscopic abnormality, it is hardly possible to miss the diagnosis. But when there is no apparent finding, uterus is accepted as normal, although endometrial cells still can be defective.

Chromoendoscopy is a widely used technique in gastrointestinal imaging [2]. Over the last decade, endoscopic systems have acquired great power due to high resolution images owing to charge-coupled device chip technology and narrow band imaging technique [3]. Besides imaging enhancement, gastroenterologic endoscopists use chemical agents either to identify specific epithelia, contrast or highlight subtle mucosal irregularities, or tattoo a specific mucosal site.

The aim of this study was to assess the value of chromohysteroscopy (endometrial dying during conven-

tional hysteroscopy) for enhancement and detection of subtle endometrial changes in recurrent IVF failure.

Materials and method

Current study was conducted between January 2001 and May 2007. Inclusion criteria were at least two consecutive IVF failures despite good-quality embryo transfer. Women with a known or detected uterine anatomical abnormality were excluded. Applying these criteria 69 women were included in the study. The ages of the cases were between 25 and 37. As this was a preliminary study, sample size was not calculated prior to the study. Institutional review board approval and informed consents were obtained.

All hysteroscopic operations were performed in the early follicular phase. In six of them hysteroscopy revealed a uterine macroscopic abnormality which was missed in ultrasonography and/or HSG (three polyp, three adhesions); they were excluded from the study. Conventional hysteroscopy did not show any apparent endometrial pathology in 63 patients. These were included in the analysis.

When no apparent abnormality was seen in the endometrial cavity, distending medium flow was stopped. Five milliliter of 1% methylene blue dye was introduced through the hysteroscopic inlet. After 5 min of waiting distending medium flow was started again and let wash the endometrium. Uterine cavity was visualized for staining pattern. Diffuse light blue staining was considered normal. Focal, dark blue staining above the internal cervical ostium, regardless of size and number of stained areas, was considered positive finding (Fig. 1). Biopsies were obtained from dark stained and light stained areas. They were sent to pathologic examination in separate bottles. When no dark stained area was seen a single biopsy was obtained from the posterior fundal region of endometrium. Pathologist was blinded during the initial histopathologic examination. After completion of the study, specimens were re-checked

by the same pathologist and the histopathologic diagnosis were confirmed.

All procedures have been performed in an out-patient setting. Classic dorsal lithotomy position is appropriated for all hysteroscopic procedures. 2.9 mm, 30° rigid telescope with an operative sheath of 3.5 mm was used for examination (Karl Storz, Germany). Neither speculum nor tenaculum was used in “no-touch technique” as described by Bettochi and Selvaggi [4]. All patients were given intraoperative antibiotics prophylaxis with 1 g ceftriaxone (Rocephin, Roche, Istanbul).

Endometrial biopsies were obtained using hysteroscopic grasping forceps. Having the jaws open, the forceps were pushed into the endometrium until sufficient tissue was grasped. There was no postoperative complication. Postoperative analgesics was required in 14 patients (22.2%); they were given diclofenac sodium (Voltaren SR 75, Novartis, Istanbul).

Time to hysteroscopy after the last IVF failure was between 29 and 192 days.

Results

Conventional hysteroscopy and chromohysteroscopy procedure was successful in all 63 patients. Prior to endometrial dying there was no apparent abnormality in any of the patients. With endometrial dying, focal dark staining areas was observed in 22 of 63 patients (34.9%), they have constituted group I. Group II included 41 patients in whom diffuse light blue staining was observed. There was no significant difference between two groups in age, smoking, body mass index, number of IVF failures and in time to hysteroscopy. Histopathologic examination revealed nine cases of endometritis in group I (40.9%). On the other hand, four cases of endometritis was diagnosed histopathologically with random biopsy in group II (9.7%). There was a statistically significant difference in the incidence of

Fig. 1 a conventional hysteroscopic view b light staining area c dark staining area

endometritis between two groups ($p=0.007$). The results are shown in Table 1.

The power of dark staining for detection of endometritis was calculated as follows: sensitivity 69.2%, specificity 74%, positive predictive value 40.9% and negative predictive value 90.2%.

Discussion

Ultrasonography, sonohysterography, three-dimensional ultrasonography and hysterosalpingography can all show uterine and corresponding endometrial anatomy. But the observation of endometrium by these tools does not assure normal endometrial lining. Hysteroscopic visualization is recommended for a direct and closer look to the endometrium in recurrent IVF failure [5]. Furthermore, a normal appearance in conventional hysteroscopy does not assure the integrity of endometrial cells. This study employed chromohysteroscopy as a new avenue to improve the diagnostic value of hysteroscopy in recurrent IVF failure.

Majority of papers in the pertinent medical literature reported macroscopic endometrial pathologies like polyp, adhesion or submucous myoma in hysteroscopy after repetitive IVF failure [5–7]. When there is a macroscopic abnormality, eventually, it is detected and treated appropriately. The value of hysteroscopy is limited for detection of subtle and/or local endometrial changes which has not produced a macroscopic finding. Endometrial biopsy is employed to diagnose cellular pathologies. La Sala et al. [8] reported an incidence of 2% endometritis among 100 women with two consecutive IVF failures. Incidence of endometritis in our series was higher. That must be related to better targeting of biopsy by endometrial dying.

Zeyneloglu et al. [9] has reported that observations of micropolyps in hysteroscopy was a significant predictor of miscarriage after IVF–ET. But, the incidence of endometritis was not stated in their abstract. Micropolyposis was

reported to be associated with endometritis in another study, too [10]. The sensitivity, specificity, positive and negative predictive values were 54%, 99%, 94% and 89% respectively. Instead of observation of micropolyposis, endometrial dying was employed in this study. Corresponding figures related to our study were different to those of Cicinelli's; most probably because of the low number of the cases included (820 vs. 63). Sensitivity, specificity, positive predictive value, negative predictive value of 69%, 74%, 40%, 90% respectively, seems quite low for a diagnostic tool, but are acceptable for screening. The novel technique of endometrial dying was not intended to be the sole procedure for diagnosis, but an adjunctive method to target the biopsy to correct site for an accurate diagnosis. Hysteroscopy, with its current abilities, can never replace pathologist's eye, pathology is still the gold standard. Broader studies and/or a new vital dye can improve the diagnostic accuracy of chromohysteroscopy.

Unlike the gastrointestinal mucosa the endometrium is not an absorptive epithelium. Endometrium is not supposed to absorb any dye, in normal circumstances. Prior to this study we had noticed that endometrium is stained by methylene blue. Various studies were designed to assess the value of staining. Later, Marconi et al. [11] also reported that endometrium is stained by methylene blue except periovulatory phase. The reason of endometrial staining is explained with apoptosis. They stated that structural damage of the cell during apoptosis would allow passage of the methylene blue dye into the cell. Dark blue staining in this study represents marked apoptotic areas due to endometritis.

It was shown that septal endometrium or endometrium covering a submucous fibroid responds suboptimally to steroid hormones and shows defective development [12]. Furthermore, local damage to endometrium as in incomplete Asherman's syndrome produces patchy fibrosis without a significant amount of intrauterine adhesions [13]. Endometrial responsiveness to steroid hormones is reduced in affected areas. It was shown that local

Table 1 Results

Characteristic	Group I $n=22$	Group II $n=41$	p
Age (years)	29.2	26.9	NS
Smoking (%)	22.7	21.9	NS
BMI (kg/m^2)	21.45	22.60	NS
Time to hysteroscopy (days)	59.30	67.50	NS
Number of failures (mean)	2.2	2.1	NS
Endometritis (%)	9 (40.9)	4 (9.7)	0.007 ^a

Comparisons were made by using Mann Whitney U test

Group I Dark staining areas, Group II diffuse light blue staining, NS not significant

^a Fisher's exact test

endometrial defects can cause miscarriage [14]. Removal of local endometrial defects may lead to replacement by healthy cells and receptivity is restored, eventually successful implantation is achieved. This theory is supported by the study of Barash et al. [15] who showed that local injury to the endometrium significantly increased the pregnancy rate in IVF. It can be speculated that, that local injury induced by a biopsy catheter might have removed the defective endometrium to be replaced by a new cell line. But the impact of focal endometritis on IVF outcome is currently unclear.

The presence of focal micro endometritis in a non IVF cycle may not mirror a stimulated endometrium. Above findings may not imply IVF failure causation. There is evidence suggesting that embryo factor and not endometrial factor is the primary cause of repeat IVF failures [16]. When oocyte donation is applied, approximately 25% of these patients will conceive [17]. But, for a woman, who is relatively young age, and with a good ovarian reserve, though not fertile, is not easy to accept oocyte donation. In a large series of 11,515 oocyte donation cycles, recurrent IVF failure constituted only 6% of the cases [18]. Recurrent IVF failure due to endometrial factor should be screened, detected and treated appropriately before referring a couple for oocyte donation.

Current study is inspired from gastroenterologists' approach to enhance subtle mucosal changes. Conventional hysteroscopy has its limitation in evaluating endometrial cell integrity. Chromohysteroscopy increased the effectiveness of the intervention. Structural endometrial cell damage as indicated by dark staining was seen in 34.9% of cases of recurrent IVF failure. Those areas contained apoptotic endometrial cells which allowed methylene blue into the cell. Same areas might have prevented either adhesion or invasion of blastocyst into the decidua properly.

One major drawback of the current study is the absence of a control group. But, offering an invasive procedure to normal women to serve as control group was found unethical by the institutional board. We could have used a control group who undergoing hysteroscopy for other causes, but that time it would not be appropriate as they might have various endometrial pathologies.

In conclusion, chromohysteroscopy improves the efficacy of hysteroscopy in recurrent IVF failure. Observation of diffuse light blue staining without dark areas strongly suggests a normal endometrium free of endometritis. Clinical application of our findings are to be determined. This preliminary study was conducted to establish the foundation of a novel tool; future modifications will refine and improve the accuracy of the technique. Broader studies are needed to draw strong conclusions and probable routine use of endometrial dying.

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