Promoting implantation by local injury to the endometrium

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Objective: To evaluate the association between endometrial injury, implantation and pregnancy rate.
Design: We performed a literature search using the keywords *endometrial injury*, *local endometrial injury*, *endometrial biopsy*, *endometrial receptivity*, *implantation*, *in vitro fertilization*, and *implantation failure* and conducted the search in Medline, EMBASE, and Cochrane Database of systematic reviews.
Setting: None.
Patient(s): None.
Intervention(s): None.
Main Outcome Measure(s): None.
Result(s): Clinical and basic science data regarding the association between endometrial injury and improved implantation rate are limited. However, current evidence suggests that endometrial injury before IVF among women with previous repeated IVF failure is associated with increased rates of implantation, clinical pregnancy, and live birth.
Conclusion(s): Endometrial injury may have a beneficial role in implantation and improve the pregnancy rate. However, there are still many unanswered question including patients selection, timing, technique and number of endometrial biopsies needed. (Fertil Steril® 2010;94:2026–9. ©2010 by American Society for Reproductive Medicine.)

Key Words: Endometrial injury, local endometrial injury, endometrial biopsy, endometrial receptivity, implantation, in vitro fertilization, implantation failure

Implantation is a process of embryonic attachment to the endometrium and subsequent invasion into the stroma of the uterine wall. It is a complex and multistage process involving several cytokines and growth factors as well as a dialogue between the embryonic tissue and the endometrium. Because implantation failure is frequent, several methods have been suggested to improve the implantation rate; however, their results have been inconsistent. One of the most promising methods is local injury to the endometrium. In 2003, Barash et al. (1) reported that endometrial injury before in-vitro fertilization (IVF) among women with repeated implantation failure was associated with increased rates of implantation, clinical pregnancy and live birth. The findings were supported by two other studies (2, 3). The purpose of our review is to examine the association between endometrial injury and implantation and discuss its possible mechanism.

INITIAL EVIDENCE

The relationship between endometrial injury and improved implantation is based on animal studies. Early studies in guinea pigs demonstrated that endometrial injury resulted in decidualization and improved receptivity of the uterus to implantation (4). The same effect was observed by injecting oil into the endometrial cavity in mice (5). The injury-induced decidualization could be prevented by administration of antihistamines into the uterine horn or by chronic treatment with chemical histamine releasers that produced depletion of endogenous histamine resources (6, 7).

A few postulations explain the improvement in the pregnancy rate after local injury. During healing of the endometrial injury, several substances are secreted including cytokines and growth factors such as leukemia inhibitory factor, interleukin-11, and heparin-binding EGF-like growth factor. These substances could facilitate implantation. Similarly, in response to local irritation by oil, the endometrium releases histamines (6–10).

Clinical Studies

In 1971, Karow et al. (11) noted that only two of 28 women who underwent endometrial biopsy in the luteal phase and who conceived in the same cycle aborted (7%). This compared favorably with the miscarriage rate in the general infertility population. They postulated that endometrial injury stimulated a better decidual reaction. Endometrial biopsy in the luteal phase however might be associated with iatrogenic miscarriage.

Two decades later, Friedler et al. (12) reported 14 patients with repeated implantation failure (>6) who were treated by a special protocol including hysteroscopy, dilation and curettage, triple antibiotics, and estrogen. Six of 14 patients conceived (pregnancy rate

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43%) in the subsequent IVF-ET cycle with implantation rate of 24%. The authors postulated that implantation failure could have been caused by endometritis that was treated with antibiotics. In addition, the endometrial flow was improved by estrogen administration. Because dilation and curettage was part of this protocol, it is also possible that endometrial injury by curettage plays a role in their improved results.

The possible role of endometrial injury on improved implantation was first emphasized by Barash et al (1); they studied 45 women who failed to conceive after one or more cycles of IVF-ET. They found that endometrial injury in the cycle before IVF significantly improved the outcome. They also postulated that the injury promotes decidualization of the endometrium making it more receptive for implantation. In their study, the endometrial injury was performed using a disposable endometrial biopsy instrument (Pipelle, Prodimed, Neuilly-en-Thelle, France) on days 8, 12, 21, and 26 of the cycle preceding IVF. The rates of implantation, clinical pregnancy, and live birth in the endometrial injury group were 28%, 67%, and 49% and in the control group were 14%, 30%, and 23%, respectively.

In a rat model, injury-induced decidualization is most effective under P influence (5). Based on this assumption, Raziel et al. (2) induced endometrial injury in the luteal phase on days 21 and 26 among 60 women with implantation failure. The mean number of previous failed IVF trials was seven. They compared the results with 57 others who did not undergo endometrial injury. The implantation and pregnancy rates in the injury group were 11% and 30%, and in the control group were 4% and 8%, respectively.

Zhou et al. (3) performed endometrial injury in women with irregular echo on ultrasound examination until the strong or homogeneous echo disappeared followed by "scratching" the endometrium once or twice. This was performed on days 5–22 of controlled ovarian hyperstimulation cycle. They also found that endometrial injury is associated with increased implantation and pregnancy rates. They postulated that local injury to the proliferative endometrium in the stimulated cycles delayed endometrial development inducing synchronicity between the endometrium and embryo stage (3). It is possible that IVF patients who fail to conceive with high-quality embryos are unable to increase the expression of genes related to endometrial receptivity in a spontaneous manner. Endometrial injury optimizes endometrial development.

Table 1 demonstrates results from three studies of local endometrial injury. There are inherent differences among them. For example, Barash et al. reported a clinical PR of 67% (1) and Raziel et al. reported 30% (2); this can be explained by the study group differences. The study group of Barash et al. had fewer previous implantation failures (mean ± SD, 4.0 ± 2.0) than that of Raziel et al. (mean ± SD, 7.0 ± 1.9). To date, there has not been a randomized study regarding this matter.

The number and time of endometrial injury are summarized in Table 1. The number of biopsies ranges one to four. As indicated previously, luteal phase–induced endometrial injury is associated with the most decidualization. However, whether endometrial injury in the luteal phase leads to a better clinical outcome than in the follicular phase is unclear. It is also unknown whether one endometrial biopsy is sufficient and whether it should be performed in the preceding or in the same stimulation cycle. Regardless, it seems that a few strokes of endometrial sampling are needed. Zhou et al. (3) used ultrasound findings as one of their criteria, and they treated their patients with antibiotics and hemostatic drugs (cefaclor and adrenobazone). Their study has some confounding factors.

Indirect Clinical Evidence

Besides the three studies to date, there have been other studies supporting the role of endometrial injury in increasing the reproductive outcome indirectly (13–15). In a randomized study of 421 women

TABLE 1

Endometrial injury and improved pregnancy rates in three published studies.

	Barash et al. (1)	Raziel et al. (2)	Zhou et al. (3)
No. of patients	45	63	60
Study design	Prospective, patient selected the treatment	Prospective, patient selected the treatment	Prospective, investigators selected the treatment
Inclusion criteria	Good responder, failed one or more trials of IVF	Good responder, failed ≥4 trials of IVF	Irregular echo by ultrasound
No. of previous failed IVF cycles, mean ± SD, range	4.0 ± 2.0, 1–9	7.0 ± 1.9, 4 –11	NA
Patient age (y), mean ± SD	33.8 ± 5.1	33.1 ± 4.9	31.6 ± 3.7
No. of endometrial biopsies	4	2	1
Timing of biopsy	Days 8, 12, 21, 26 of preceding cycle	Days 21, 26 of preceding cycle	Once in day 5–22 of the treatment cycle
No. of embryos transferred, mean ± SD	3.4 ± 1.0	3.3 ± 0.9	2.2 ± 0.5
Implantation rate (%), study vs. control group	27.7 vs. 14.2	11.0 vs. 4.0	33.3 vs. 17.7
Clinical pregnancy rate (%), study vs. control group	66.7 vs. 30.3	30.0 vs. 12.0	48.3 vs. 27.8
Live birth rate (%), study vs. control group	48.9 vs. 22.5	22.0 vs. 8.0[a]	41.6 vs. 22.9[b]

Note: NA = not available.
[a] Ongoing pregnancy rate.
[b] Ongoing pregnancy rate or live birth rate.

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who underwent two or more failed IVF cycles, the authors randomly assigned the patients into two groups; group I (n = 211) did not have hysteroscopy, and group II (n = 210) had hysteroscopy examination (13). Group II was further divided into group IIa (n = 154, normal hysteroscopy findings) and group IIb in which the abnormal hysteroscopic findings were corrected (n = 56). The clinical pregnancy rates in groups I, IIa, and IIb were 21.6%, 32.5%, and 30.4%, respectively. There was a significant difference in the clinical pregnancy rates between patients in groups I and IIa (21.6% and 32.5%, respectively) and groups I and IIb (21.6% and 30.4%, respectively). However, there was no significant difference in the clinical pregnancy rate of patients in groups IIa and IIb. It appears that hysteroscopy per se was the only factor that increased the subsequent implantation rate. Hysteroscopy induces some injury to the endometrium. Similar findings were reported by Rama Raju et al. (15) and Mooney et al. (14). They found that hysteroscopy increased the pregnancy rate regardless of the findings.

Basic Science Support

Basic scientific studies regarding the pathophysiology of local injury and improved implantation are lacking. The only study to date investigating endometrial gene modulation following endometrial injury was performed by Kalma et al. (16). They compared two groups of patients: the study group underwent two endometrial biopsies in one menstrual cycle, and the control group did not undergo any biopsy. In the following cycle, both groups underwent endometrial biopsy on day 21 of the cycle, followed by IVF-ET treatment. Day 21 endometrial samples of four patients from the study group who conceived were compared with four others in the control group who did not conceive. By profiling global gene expression using microarray analysis, they found 2- to 10-fold increases in the expression of 183 genes in the endometrial samples of the biopsy-treated patients. The expression of 39 genes in these samples was downregulated by at least twofold. Genes that were upregulated in the endometrial samples of the biopsy-treated patients included mucin 1 transmembrane (MUC1), crystallin alpha B, apolipoprotein D (APOD), phospholipase A2 (PLA2), and uroplakin Ib (UPIb), which had the highest upregulation. These genes seem to be involved in the preparation of the endometrium for implantation (17, 18), supporting the hypothesis that local injury increases endometrial receptivity by modulating the expression of a variety of genes. Their findings suggest that endometrial injury modulates expression of a wide variety of genes. Phospholipase A2 is among the highest upregulated genes in women who underwent endometrial biopsy (16). Song et al. (19) found that in women lacking phospholipase A2, the initiation of implantation was deferred, shifting the normal window of implantation and leading to retarded fetoplacental development. Dey et al. (20, 21) concluded that the PLA2 pathway is crucial for implantation.

MUC1 is expressed in the endometrium both in the proliferative and secretory phases of the cycle (22). Its expression increases from the secretory phase of the cycle throughout early pregnancy in response to high blood progesterone levels (23). There is also a significant increase in the concentration of MUC1 in the uterine flushings from day 7 after the LH peak (24). This is the time that implantation would be expected to occur in a conception cycle. MUC1 represents a potential ligand for selectins that are known to be expressed by human blastocysts, and which may have an important role in the adhesion of the blastocyst to the endometrium (25). Similar to phospholipase A2, MUC1 was also among the highest upregulated genes in biopsy-treated women (16).

Endometrial gene profile expression in natural cycles is dynamic and gene expression at different time of the cycle varies (17, 18). Many genes yield at least a threefold increase between early luteal phase (day 2 of luteal phase) and the implantation day (day 7 of luteal phase). The strongest transcriptional upregulation (107-fold) is for glycodelin A (GdA). Other studies have also demonstrated upregulation of this gene and suggest a possible role for GdA in the implantation window (26).

Endometrial gene expressions in natural and stimulated cycles are different. For example, GdA expression in the IVF cycle decreased by 9.8-fold, whereas in the spontaneous cycle it increased 107-fold (18). Endometrium in IVF cycles is ahead of that of natural cycle by 2–4 days. It is possible that repeated IVF-ET implantation failure is related to asynchrony of the endometrium with the embryo stage (27–31). Zhou et al. (3) postulated that local endometrial injury in stimulated cycle delays the endometrial development because of wound repair processes correcting the asynchrony between endometrial and embryo stage.

CONCLUSIONS

Evidence to date suggests that local endometrial injury might improve the pregnancy rate. However, there are still many unanswered questions regarding patient selection, timing, technique, and number of biopsies needed.

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