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Effect of endometrial injury on implantation and pregnancy rates: a randomised controlled trial

S.A. Hebeisha, F.S. Moiety, M. Samir, M. Hussein

Department of Obstetrics and Gynecology, Faculty of Medicine, University of Alexandria, Alexandria (Egypt)

Summary

Objective: To study the effect of induced endometrial injury on the pregnancy and implantation rates in patients undergoing intracytoplasmic sperm injection (ICSI) cycles. *Study Design:* Randomised case control study. *Setting:* University hospital's fertility clinic. *Materials and Methods:* A total of 240 women undergoing ICSI cycles were randomly divided into three equal groups, Group A underwent endometrial scratching by pipelle, group B underwent hysteroscopic endometrial injury, and group C were controls. *Results:* No difference was seen between the groups in terms of age, BMI, endometrial thickness, number of oocytes retrieved, or number of embryos transferred. Pregnancy rate was similar in groups A and B, which was higher than group C, but the difference was not statistically significant. Implantation rate was significantly higher in both groups A and B than controls, and significantly in group B compared to group A. *Conclusions:* Endometrial injury, especially if induced by hysteroscopy, might be helpful to increase the implantation rate in cases undergoing ICSI-embryo transfer (ICSI-ET). Pregnancy rate however did not change.

Key words: Endometrium; Injury; Pregnancy; Implantation.

Introduction

Implantation is a process of attachment and invasion of the endometrium by the blastocyst (conceptus) in placental animals. In human race, this conceptus implants almost eight to ten days after ovulation [1]. Recurrent implantation failure is when transferred embryos do not implant, or reach a recognizable stage by ultrasonography after many in vitro fertilization (IVF) treatment cycles. This may be due to: suboptimal embryo quality, poor endometrial receptivity, immunological factors, abnormal uterine, tubal and/or peritoneal factors, and causes related to culture media. Any abnormality attributed to the embryo, the endometrium or the immune system will result in implantation failure. The embryo itself is responsible for only one-third of implantation failures [2]. Abnormal embryo karyotyping might explain failure of implantation and miscarriage. Endometrial receptivity includes a series of events that make the endometrium receptive to the embryo [3]. It represents the window of time when the uterine cavity accepts blastocyst conduction followed by implantation [4]. It has been suggested that patients may benefit from mechanical endometrial stimulation (e.g. by means of an endometrial biopsy catheter) performed in the cycle preceding the treatment cycle, which may induce an inflammatory response, thus facilitating the preparation of the endometrium for implantation [5]. Locally secreted cytokines control the implantation process and can cause implantation failure [6]. IL-6 family, is of great importance for the implantation process [7]. Leukaemia inhibitory factor (LIF) is involved and has a role in implantation, especially during the endometrial receptive state, embryo-endometrial interaction, and stromal decidualization. LIF has also been found to play an important role in regulating synthesis of prostaglandins (PGs), an important mediator of implantation and decidualization [8-10]. Increased production of growth factors, such as epidermal growth factor (EGF), heparin binding-epidermal growth factor (HB-EGF), and vascular endothelial growth factor (VEGF) was detected during the time of high endometrial receptivity, and might maintain a role in both adhesion and development in the embryo [11, 12]. The release of some or all of the aforementioned factors might favourably and sustainably assist in the implantation success. This may be achieved by a simple manoeuvre such as endometrial sampling catheter or pipelle, or via hysteroscopy. The latter might have the advantage of being more sensitive, specific, and accurate in evaluating uterine pathology for patients with recurrent implantation failure [13]. It has been strongly suggested that the use of hysteroscopy for repeated IVF failure may lead to a significant success of on-going pregnancy [14]. Even in the absence of intrauterine pathology, hysteroscopy may enhance fertility chances based on the fact that cervical dilatation and/or direct hysteroscopic visualization of the uterine cavity facilitates embryo transfer [15]. Moreover, an immunological mechanism triggered by the hysteroscopic manipulation, or by the effect of the distension medium on the endometrium, might play a role [16]. In this

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Table 1. — *Patients' data analysis in terms of age, BMI, endometrial thickness, oocytes retrieved, and embryos transferred.*

		Group A (n = 60)	Group B (n = 60)	Group C (n = 60)	F	*p*
Age (years)	Min – max	22.0 – 40.0	20.0 – 40.0	20.0 – 40.0	1.332	0.267
	Mean ± SD	29.67 ± 4.55	29.3 ± 5.19	30.87 ± 5.69		
	Median	29.0	29.0	30.50		
BMI (kg/m^2)	Min – max	24.0 – 44.0	20.03 – 40.80	20.10 – 43.30	0.338	0.714
	Mean ± SD	28.0 ± 4.18	28.34 ± 4.50	27.67 ± 4.70		
	Median	27.48	27.50	26.70		
Endometrial thickness	Min – max	9.0 – 15.0	9.0 – 15.0	9.0 – 16.0	F = 2.307	0.103
	Mean ± SD	11.23 ± 1.23	11.58 ± 1.14	11.67 ± 1.17		
	Median	11.0	11.50	11.50		
Number of oocytes	Min – max	4.0 – 30.0	4.0 – 30.0	5.0 – 30.0	$^{KW}\chi^2 = 4.004$	0.135
	Mean ± SD	20.0 ± 7.36	21.13 ± 7.46	22.40 ± 7.96		
	Median	20.50	20.0	25.50		
Number of embryos	Min – max	1.0 – 3.0	2.0 – 3.0	2.0 – 3.0	2.717	0.069
	Mean ± SD	2.85 ± 0.44	2.88 ± 0.32	2.98 ± 0.13		
	Median	3.0	3.0	3.0		

F: F test (ANOVA); $^{KW}\chi^2$: Chi square for Kruskal Wallis test.

study, the effect of endometrial injury before ICSI on implantation and pregnancy rates was evaluated.

Materials and Methods

Approval of the official ethical committee board was granted before commencing this research. A total of 240 infertile women undergoing ICSI cycles were recruited during the period from January 2015 to October 2015. All participants signed a full informed written consent, and underwent a physical examination and detailed medical history obtaining. They were then randomly divided into three equal groups, using the online researcher randomizer software (www.randomizer.org/form.htm): group A, B, and C. Group A (n=80) underwent endometrial injury during the mid-luteal phase of the previous cycle, performed by the first author, using endometrial pipelle, on a basis of two longitudinal anterior and two posterior scratches. Group B (n=80), underwent office hysteroscopy by one surgeon (second author), using normal saline distension, and the vaginoscopic approach. The authors used a 2.9-mm semi-rigid hysteroscope and any abnormality was recorded to exclude the case. A hysteroscopic grasper was then used to perform a blunt endometrial scratching, on the basis of two longitudinal anterior and two posterior scratches. Group C (n=80) was control.

All cases then underwent downregulation of ovaries by gonadotrophin releasing hormone agonist (0.1 mg/d, subcutaneously) from day 21 of previous cycle. They had controlled ovarian stimulation (COS) by a fixed daily dose of 225 IU hMG. Ovulation induction (OI) was planned on the basis of maturity of follicles. hCG injection was given approximately 14 days post-GnRH agonist regimen or when follicles reached from 16 to 18 mm in size. Intracytoplasmic sperm injection and embryo transfer (ICSI/ET) was performed on day 3.

Outcome measures: implantation rate (calculated as the number of intrauterine gestational sacs observed by transvaginal ultrasonography divided by the number of transferred embryos), and pregnancy rate, based on serum ß-hCG) > 25 and cardiac activity on transvaginal ultrasound scan.

Table 2. — *Comparison between the studied groups according to pregnancy rate.*

	Group A (n = 60) No. %	Group B (n = 60) No. %	Group C (n = 60) No. %	χ^2	*p*
Pregnancy					
-ve	24 40.0	24 40.0	35 58.3	5.411	0.67
+ve	36 60.0	36 60.0	25 41.7		

χ^2: Chi square test; *: statistically significant at $p \leq 0.05$.

Data were fed to the computer and analysed using SPSS software package version 20.0. Qualitative data were described using number and percentage. Quantitative data were described using range, mean, standard deviation, and median. Comparison between categorical variables was tested using Chi-square test. Significance of the obtained results was judged at the 5% level. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test [17, 18].

Results

A total of 240 patients undergoing ICSI were studied. Neither cycle cancellation nor dropouts were reported. No difference was seen between the groups in terms of age, body mass index (BMI), endometrial thickness, number of oocytes retrieved, or number of embryos transferred (Table 1). Pregnancy rate was similar in groups A and B, which was higher than group C, but the difference was not statistically significant (Table 2). Implantation rate was significantly higher in both groups A and B than controls, and significantly in group B compared to group A (Table 3, Figure 1).

Table 3. — *Comparison between the studied groups according to implantation rate.*

	Group A	Group B	Group C	χ^2	*p*
No. of fetuses	54	90	40		
Total no. of embryos transferred	171	173	179	16.505*	<0.001*
Implantation rate	31.6%	52.0%	22.3%		
	p_1<0.001*, p_2<0.001*, p_3<0.001*				

χ^2: Chi square test; p_1: *p* value for comparing between group A and B;
p_2: *p* value for comparing between group A and C;
p_3: *p* value for comparing between group B and C;
*: statistically significant at $p \leq 0.05$.

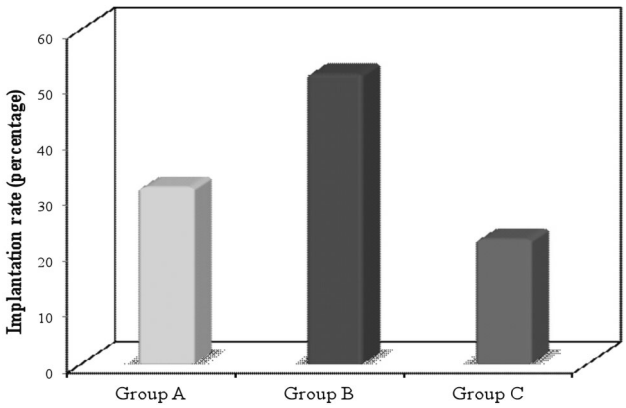

Figure 1. — Comparison between the studied groups according to implantation rate.

Discussion

Local endometrial injury (EI), whether in the same or preceding cycle, using a hysteroscope or an endometrial sampling catheter, has been observed in many reviews as a reasonable procedure to optimize the reproductive outcome of IVF/ICSI, particularly for women with previous unsuccessful embryo transfer [19 -22].

The present authors demonstrated a significant positive effect of EI done in the preceding cycle on implantation rate, which was confirmed in group A (EI using endometrial pipelle), and group B (EI using hysteroscope) versus control. The results also confirmed a higher pregnancy rate in group A and B, than in group C, however, it did not reach a level of statistical significance.

Barash *et al.* induced EI four times in the spontaneous cycle (days 8, 12, 21, and 26), and reported matching findings to the present results, confirming a possible positive impact on implantation and pregnancy rates in the biopsy-treated patients versus control [23].

Raziel *et al.* reported a group of 60 patients, for whom they performed a pipelle biopsy twice on days 21 and 26 in the preceding cycle before ICSI. They demonstrated an increased implantation, clinical pregnancy, and ongoing pregnancy rates in the intervention groups [24]. There has been no data in support of a correlation between endometrial thickness and pregnancy rates [25, 26]. The present authors did not find any correlation between the two variables. Liang *et al.* reported similar results to the current work, however, they found a significant improvement in the clinical pregnancy rate, with a non-significantly higher implantation rate [27]. In addition, Karimzade *et al.* performed EI on the day of oocyte retrieval in women undergoing the first IVF cycle, and reported contradicting findings to the present results, where a negative impact on implantation and IVF outcomes were claimed by the procedure [28]. Yeung *et al.* also reported a non-significant differences in the implantation, clinical pregnancy, live birth, multiple pregnancy, or the ongoing pregnancy rates between the EI group and control, They claimed the patient's age to be the most important factor affecting the ongoing pregnancy [29].

Many published reports were similar to the current study, emphasizing a positive role for endometrial injury on clinical pregnancy or implantation rates or live birth rate [30-34]. This might be due to cytokines and other growth factors' local release under the influence of endometrial injury, which would remain at the basal endometrium for a few cycles and enhance decidualization, and thus facilitate implantation. In addition, EI may be responsible for endometrial development process, through upregulation of endometrial receptivity-related gene expression. Another hypothesis in favour of the present findings, was published by Huang *et al.* who confirmed that a mechanical injury may enhance uterine receptivity, and if the immune system was stimulated with an injury, the immune and inflammatory response may in turn promote the endometrial receptivity to the implanting embryo [35].

Conclusion

The present authors believe, that endometrial injury, bluntly induced by hysteroscopy, or via an endometrial sampling pipelle, might be helpful to increase the implantation rate in cases undergoing an ICSI-ET procedure the cycle after. Pregnancy rate did not significantly change. Further research is still needed to verify these results.

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References

[1] Wilcox A.J., Baird D.D., Weinberg C.R.: "Time of implantation of the conceptus and loss of pregnancy". *N. Engl. J. Med.*, 1999, *340*, 1796.

[2] Achache H., Revel A.: "Endometrial receptivity markers, the journey to successful embryo implantation". *Hum. Reprod. Update*, 2006, *12*, 731.

[3] Bergh P.A., Navot D.: "The impact of embryonic development and endometrial maturity on the timing of implantation". *Fertil. Steril.*, 1992, *58*, 537.

[4] Swierz L., Giudence L.: "Unexplained infertility and the role of uterine receptivity". *Clinics North America*, 1997, *8*, 523.

[5] Gnainsky Y., Granot I., Aldo P.B., Barash A., Or Y., Schechtman E., *et al.*: "Local injury of the endometrium induces an inflammatory response that promotes successful implantation". *Fertil. Steril.*, 2010, *94*, 2030.

[6] Lédée-Bataille N., Laprée-Delage G., Taupin J.L., Dubanchet S., Frydman R., Chaouat G.: "Concentration of leukaemia inhibitory factor (LIF) in uterine flushing fluid is highly predictive of embryo implantation". *Hum. Reprod.*, 2002, *17*, 213.

[7] Dimitriadis E., White C.A., Jones R.L., Salamonsen L.A.: "Cytokines, chemokines and growth factors in endometrium related to implantation". *Hum. Reprod. Update*, 2005, *11*, 613.

[8] Cullinan E.B., Abbondanzo S.J., Anderson P.S., Pollard J.W., Lessey B.A., Stewart C.L.: "Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/ paracrine function in regulating embryo implantation". *Proc. Natl. Acad. Sci. U S A*, 1996, *93*, 3115.

[9] Wang W., Taylor R.N., Bagchi I.C., Bagchi M.K.: "Regulation of human endometrial stromal proliferation and differentiation by C/EBPβ involves cyclin E-cdk2 and STAT3". *Mol. Endocrinol.*, 2012, *26*, 2016.

[10] Horita H., Kuroda E., Hachisuga T., Kashimura M., Yamashita U.: "Induction of prostaglandin E2 production by leukemia inhibitory factor promotes migration of first trimester extravillous trophoblast cell line, HTR-8/SVneo". *Hum. Reprod.*, 2007, *22*, 1801.

[11] Birdsall M.A., Hopkisson J.F., Grant K.E., Barlow D.H., Mardon H.J.: "Expression of heparin-binding epidermal growth factor messenger RNA in the human endometrium". *Mol. Hum. Reprod.*, 1996, *2*, 31.

[12] Tamada H., Higashiyama C., Takano H., Cohen J., Massey J.B., Robinson J., *et al.*: "The effects of heparin-binding epidermal growth factor-like growth factor on preimplantation-embryo development and implantation in the rat". *Life Sci.*, 1999, *64*, 1967.

[13] Arefi S., Soltanghoraei H., Sadeghi M.R., Tabaei A.S., Zeraati H., Zarnani H.: "Findings on hysteroscopy in patients with in vitro fertilization by intra cytoplasmic single sperm injection and embryo transfer failures". *Saudi Med. J.*, 2008, *29*, 1201.

[14] Makrakis E., Hassiakos D., Stathis D., Vaxevanoglou T., Orfanoudaki E., Pantos K.: "Hysteroscopy in women with implantation failures after in vitro fertilization: findings and effect on subsequent pregnancy rates". *J. Minim. Invasive Gynecol.*, 2009, *16*, 181.

[15] Mansour R., Aboulghar M.: "Optimizing the embryo transfer technique". *Hum. Reprod.*, 2002, *17*, 1149.

[16] Luttjeboer F., Harada T., Hughes E., Johnson N., Lilford R., Mol B.W.: "Tubal flushing for subfertility". *Cochrane Database Syst. Rev.*, 2007, *3*, CD003718.

[17] Leslie D., Geoffrey B.: "Statistical analysis. Interpretation and uses of medical statistics". 4th ed. Oxford: Oxford Scientific Publications, 1991.

[18] Kirkpatrick L., Feeney B.: "A simple guide to IBM SPSS statistics for version 20.0". Student ed. Belmont. CA: Wadsworth Publishing, 2013, 12, 85

[19] Nastri C.O., Gibreel A., Raine-Fenning N., Maheshwari A., Ferriani R.A., Bhattacharya S., *et al.*: "Endometrial injury in women undergoing assisted reproductive techniques". *Cochrane Database Syst. Rev.*, 2012, *7*, CD009517.

[20] El-Toukhy T., Sunkara S., Khalaf Y.: "Local endometrial injury and IVF outcome: A systemic review and meta-analysis". *Reprod. Biomed. Online*, 2012, *25*, 345.

[21] Potdar N., Gelbaya T., Nardo L.G.: "Endometrial injury to overcome recurrent embryo implantation failure: A systematic review and meta-analysis". *Reprod. Biomed. Online*, 2012, *25*, 561.

[22] Pundir J., Pundir V., Omanwa K., Khalaf Y., El-Toukhy T.: "Hysteroscopy prior to thefirst IVF cycle: A systematic review and meta-analysis". *Reprod. Biomed. Online*, 2014, *28*, 151.

[23] Barash A., Dekel N., Fieldust S., Segal I., Schechtman E., Granot I.: "Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization". *Fertil. Steril.*, 2003, *79*, 1317.

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

[25] Chen S.L., Wu F.R., Luo C., Chen X., Shi X.Y., Zheng H.Y.: "Combined analysis of endometrial thickness and pattern in predicting outcome of in vitro fertilization and embryo transfer: a retrospective cohort study". *Reprod. Biol. Endocrinol.*, 2010, *8*, 30.

[26] Barker M.A., Boehnlein L.M., Kovacs P., Lindheim S.R.: "Follicular and luteal phase endometrial thickness and echogenic pattern and pregnancy outcome in oocyte donation cycles". *J. Assist. Reprod. Genet.*, 2009, *26*, 243.

[27] Liang Y., Han J., Jia C., Ma Y., Lan Y., Li Y.: "Effect of Endometrial Injury on Secretion of Endometrial Cytokines and IVF Outcomes in Women with Unexplained Subfertility". *Mediators Inflamm.*, 2015, *2015*, 757184.

[28] Karimzade M.A., Oskouian H., Ahmadi S., Oskouian L.: "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.*, 2010, *281*, 499.

[29] Yeung T.W.Y., Chai J., Li R.H.W., Lee V.C.Y., Ho P.C., Yu Ng E.H.: "The effect of endometrial injury on ongoing pregnancy rate in unselected subfertile women undergoing in vitro fertilization: a randomized controlled trial". *Hum. Reprod.,* 2014, *29*, 2474.

[30] Almog B., Shalom-Paz E., Dufort D., Tulandi T.: "Promoting implantation by local injury to the endometrium". *Fertil. Steril.*, 2010, *94*, 2026.

[31] Kalma Y., Granot I., Gnainsky Y., Or Y., Czernobilsky B., Dekel N., *et al.*: "Endometrial biopsy-induced gene modulation: First evidence for the expression of bladder transmembranal uroplakin Ib in human endometrium". *Fertil. Steril.*, 2009, *91*, 1042.

[32] Gibreel A., Badawy A., El-Refai W., El-Adawi N.: "Endometrial scratching to improve pregnancy rate in couples with unexplained subfertility: a randomized controlled trial". *J. Obstet. Gynaecol. Res.*, 2013, *39*, 680.

[33] Shohayeb A., El-Khayat W.: "Does a single endometrial biopsy regimen (S-EBR) improve ICSI outcome in patients with repeated implantation failure? A randomised controlled trial". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2012, *164*, 176.

[34] Nastri C.O., Ferriani R.A., Raine-Fenning N., Martins W.P.: "Endometrial injury performed in the non-transfer cycle and assisted reproduction outcomes: a randomized controlled trial". *Ultrasound Obstet. Gynecol.*, 2013, *42*, 375.

[35] Huang S.Y., Wang C.J., Soong Y.K., Wang H.S., Wang M.L., Lin C.Y., Chang C.L.: "Site-specific endometrial injury improves implantation and pregnancy in patients with repeated implantation failures". *Reprod. Biol. Endocrinol.*, 2011, *9*, 140.

Corresponding Author:
F.S. MOIETY, M.D.
Shatby University Hospital
Obstetrics & Gynecology Department
Portsaid Street
Shatby, Alexandria 21526 (Egypt)
e-mail: fmoiety@gmail.com