

Original Research Article

Effectiveness of Endometrial Scratch in Enhancement of Endometrial Receptivity Marker and Pregnancy Outcome in Intra Cytoplasmic Sperm Injection Cycles

Fadya Kadhim Al-Jorani, Manal Taha Al-Obaidi, Wasan Adnan Abdulhameed and
Mohammad Oda Selman*

Abstract

High Institute of Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University

*Corresponding Author's E-mail:
mohammadoda@yahoo.com

The goal of our trial was to determine whether endometrial injury in luteal phase of the preceding cycle to IVF cycles would improve pregnancy rates through its effect on vascular endothelial growth factor level and endometrial thickness. This prospective study was undertaken in the Higher Institute of Infertility diagnosis and Assisted Reproductive Techniques/Al-Nahrain University. All women had undergone Intra Cytoplasmic Sperm Injection cycles. Endometrial scratching had been done for the females in the intervention group with a pipelle catheter in luteal phase prior to ICSI cycle. Blood sample was taken before scratch. All patients undergo controlled ovarian hyper stimulation, oocyte retrieval and embryo transfer. At this day, another blood sample was taken for the measurement of the level of VEGF and ultrasound used to measure endometrial thickness. Two weeks later clinical pregnancy rate were evaluated after luteal support. The patients in scratch group had higher pregnancy rate (14/23) 60.9% than non-scratched group (11/33) 33.3% although not significant (p value 0.057). Also these tests revealed that there were significant differences in vascular endothelial growth factor level P value (>0.00) in scratch group which is higher than before the procedure (279.86 ± 126.0 , 134.55 ± 64.07). Furthermore, the VEGF level in scratch group (279.86 ± 126.0) is significantly higher than control group (208.35 ± 72.25) as p value was (0.020). The endometrial thickness of the scratch group (1.0 ± 0.13) was significantly higher than non-scratched (0.92 ± 0.12) as p value (0.03). It is safe and simple to use endometrial injury induced by pipelle biopsy as an outpatient procedure which is associated with significant improvement in implantation, by providing a favorable environment which enhance endometrium and sub endometrium vascularization of the uterus. Also it improves endometrial thickness.

Keywords: Pipelle biopsy, Endometrial scratching and VEGF

INTRODUCTION

In many cases of male and female infertility, the assisted reproductive techniques (ART's) have been become the preferable choice for the treatment of these cases, and

despite the progression which had been occurred in in-vitro-fertilization (IVF) technology but the rates of success of these technologies are still relatively low. ART's have

conquered numerous defects in the fertility, but the implantation still considered a limited step for the successful rates of IVF (Nordqvist, 2016). The opposition of the blastocyst to the endometrium is the beginning of embryo implantation process, followed by the attachment of the embryo to the surface epithelium of the endometrium. In order the implantation to takes place, a receptive uterus should be present. Which becomes more receptive during the mid-secretory phase (days 19–23) of the menstrual cycle, named by window of implantation (Dekel et al., 2014). For the occurrence of the implantation, a genetically normal blastocyst must be able to hatch, appose, adhere, penetrate, and lastly invade a well-synchronized endometrium, under the effect of estrogens and progesterone (Narvekar et al., 2010). Numerous studies and researches is taking place to conquer the hurdle of implantation. Finally scratching of endometrium before embryo transfer (ET) is being investigated as a possible technique to increase implantation rate. Endometrial scratching and its beneficial effect on implantation were first studied by Barash et al. in 2003. They demonstrated that endometrial biopsy done in the luteal phase of the menstrual cycle is associated with higher pregnancy rate after IVF (Barash et al., 2003). They also hypothesized that scratching to the endometrium may enhance secretion of growth factors and cytokines within the process of wound healing, by which the implantation could be helped. This scratching improve the receptivity and clinical pregnancy rates of IVF-ET by three possible ways, the implantation probability increases by enhancement of decidualization due to scratching (Li and Hao, 2009). The better synchronicity between endometrium and transferred embryo which can be achieved by scratching when done to the endometrium in the previous cycle (Alijotas-Reig and Garrido-Gimenez, 2013) might overcome the abnormal advanced maturation of the endometrium when controlled ovarian stimulation is performed during ART, (Nava et al., 2010; Van Der Gaast et al., 2012). Finally a significant increase in the secretion of cytokines, interleukins, growth factors, migration of macrophages, and dendritic cells, which occur during the healing following endometrial injury, all of that have beneficial effect on embryo implantation (Li and Hao, 2009; Alijotas-Reig and Garrido-Gimenez, 2013).

VEGF is a signaling protein that promotes the growth of new blood vessels. It forms a part of the mechanism which restores the blood supply to cells and tissues when they deprived of oxygenated blood due to compromised blood circulation (Hoeben et al., 2004).

The remodeling of vascularity of the endometrium takes place during menstruation and endometrial differentiation processes. The angiogenic factor, vascular endothelial growth factor (VEGF)-A crucially regulate the permeability of the vessels and also performing the formation of new blood vessels by controlling and

enhancement of proliferation of endothelial cells, migration, differentiation in the endometrium and maintenance of vessel fragility (Sen et al., 2002).

A little suspicion demonstrates that a physiologically thickened endometrium is important to a successful implantation and pregnancy. The thickened endometrium provides a site for attachment, and is the source of nourishment for an implanting embryo during its first weeks of pregnancy, until the time of fully placenta maturation (Kovacs et al., 2003). A correlation between endometrial thickness and receptivity, showing significantly greater endometrial thickness for pregnancy versus no pregnancy cycles (Kovacs et al., 2003). This relationship between endometrial thickness and pregnancy rate is obviously giving another clue to suggest that endometrial thickness is a useful indicator of endometrial receptivity. Studies showed significant differences in the rate of pregnancy above and below a threshold thickness of 8–10 mm, but did not suggest a continuous relationship (Zhang et al., 2005; Smith, 2011).

MATERIALS AND METHODS

A randomized prospective study was conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, from October 2017 to April 2018. Fifty six women were included in this study.

These fifty six infertile women of age range from 18-39 years with no congenital or pathological (polyp or fibroid) uterine anomalies undergoing ICSI-ET cycles were randomized into two groups. Intervention group: 23 ICSI patient women had undergone endometrial scratching once between days 19–23 of Oral Contraceptive Pills menstrual cycle (in the cycle prior to ICSI) and blood samples taken to measure TNF-alpha
Control group: 33 ICSI patient women were the scratching had not been done.

For both groups either long agonist protocol or antagonist protocol for COH was used, follow up by ultrasound till at least 3 follicles reaching a size of 17–18 mm, were hCG trigger was given. 34-36 hours later ova-pickup was done, 2-3 days later transfer of at least one grade one embryo.

At day of embryo transfer, for both groups blood samples were taken to measure the levels of VEGF in serum, ultrasound to measure the endometrial thickness.

Fourteen days later, B-HCG test was done in blood to check pregnancy.

RESULTS

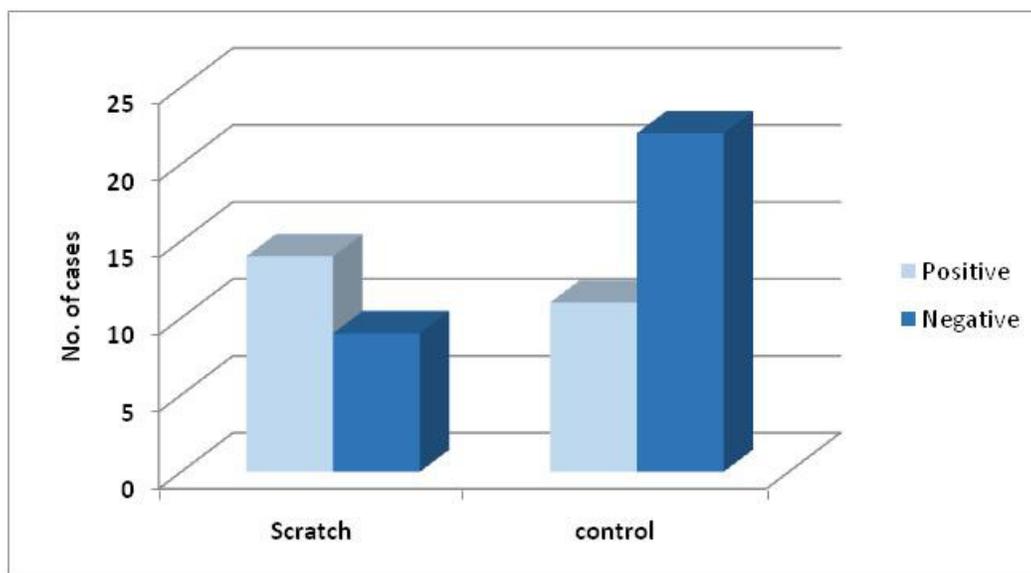
Demographic parameters

Regarding the demographic parameters, the statistical

Table 1. Comparison of demographic data between scratch and control groups

| Parameters | | Scratch group N=23 mean±SD | Control group N=33 mean±SD | P value |
|--------------------------------------|---------------|----------------------------------|----------------------------------|---------|
| Age (yr) | | 29.74±5.88 | 29.55±5.55 | 0.902* |
| Body mass index (kg/m ²) | | 27.55±4.58 | 29.08±3.46 | 0.185* |
| Duration of infertility (yr) | | 6.65±3.24 | 8.3±4.67 | 0.124* |
| | | No. (%) | No. (%) | |
| No. of trials | 1 | 19 (82.6) | 33 (100) | 0.284** |
| | 2 | 3 (13.0) | 0 (0) | |
| | 3 | 1 (4.4) | 0 (0) | |
| Cause of infertility | Combined | 9 (39.1) | 4 (12.1) | 0.073** |
| | Female factor | 2 (8.7) | 13 (39.4) | |
| | Male factor | 11 (47.8) | 14 (42.4) | |
| | Unexplained | 1 (4.4) | 2 (6.1) | |
| Type of infertility | Primary | 16 (69.6) | 25 (75.8) | 0.761** |
| | Secondary | 7 (30.4) | 8 (24.2) | |

*unpaired t-test, ** Yates chi square

**Figure 1.** Pregnancy rate in scratch and control group

analysis showed that there are no significant differences between scratch and control groups of ICSI patients, concerning age, BMI of the patients and duration of infertility, number of trials, cause of infertility, type of infertility, p value (0.902, 0.185, 0.124, 0.284, 0.073, 0.761 respectively), as in table (1)

Pregnancy Rate

Despite of non-significant results of pregnancy rate between the two groups, p value (0.057). Out of 23 patients with endometrial scratching 14 patients get pregnancy (60.9%), while we have 11 out of 33 patients

with control group get pregnancy (33.3%) as shown in figure 1.

Endometrial Receptivity parameters

Vascular endothelial growth factor (VEGF)

These tests revealed that, there are significant differences in VEGF level P value (0.001>) in scratch group which is higher after than before the procedure (279.86±126.0, 134.55±64.07) respectively.

Furthermore, the VEGF level in scratch group (279.86±126.0) is significantly higher than control group

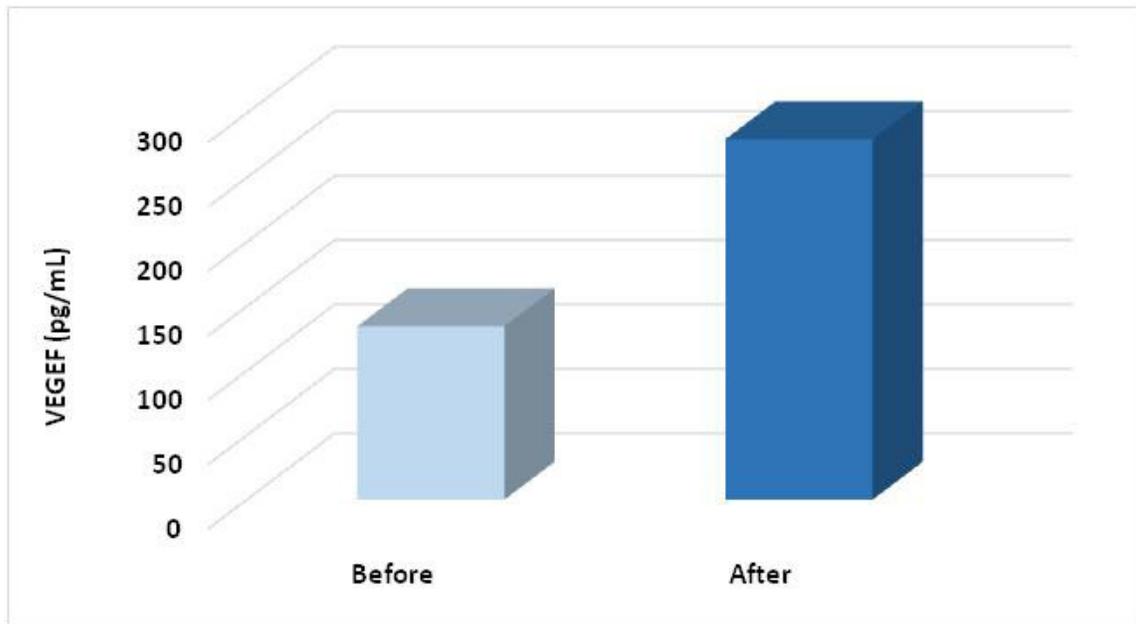


Figure 2. Comparison of VEGF before and after scratching

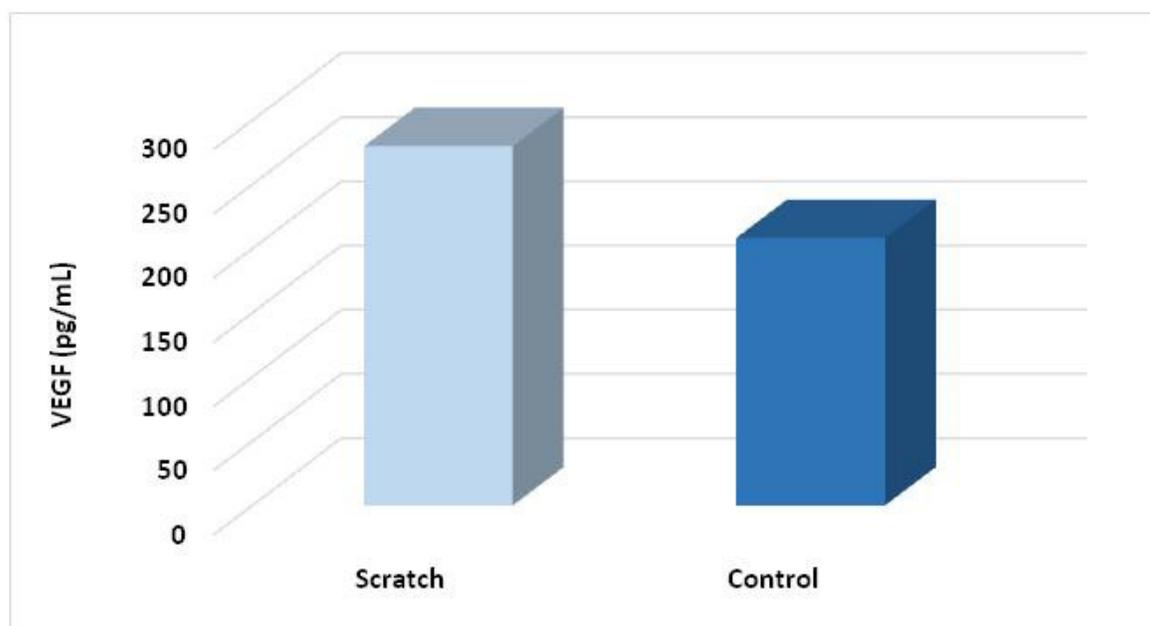


Figure 3. Comparison of VEGF between scratch and control groups

(208.35 ± 72.25) as p value was (0.020), as shown in Figure 2, 3.

Endometrial Thickness

The endometrial thickness (1.0 ± 0.13) was significantly higher than non-scratched (0.92 ± 0.12) as p value (0.03) (Figure 4).

Correlation between VEGF and endometrial thickness

Regarding the correlation between the endometrial thickness of the patients at day of embryo transfer and the level of VEGF at the same day, the figure 5 showed a significant, positive correlation. $r = 0.200$, p value = 0.35

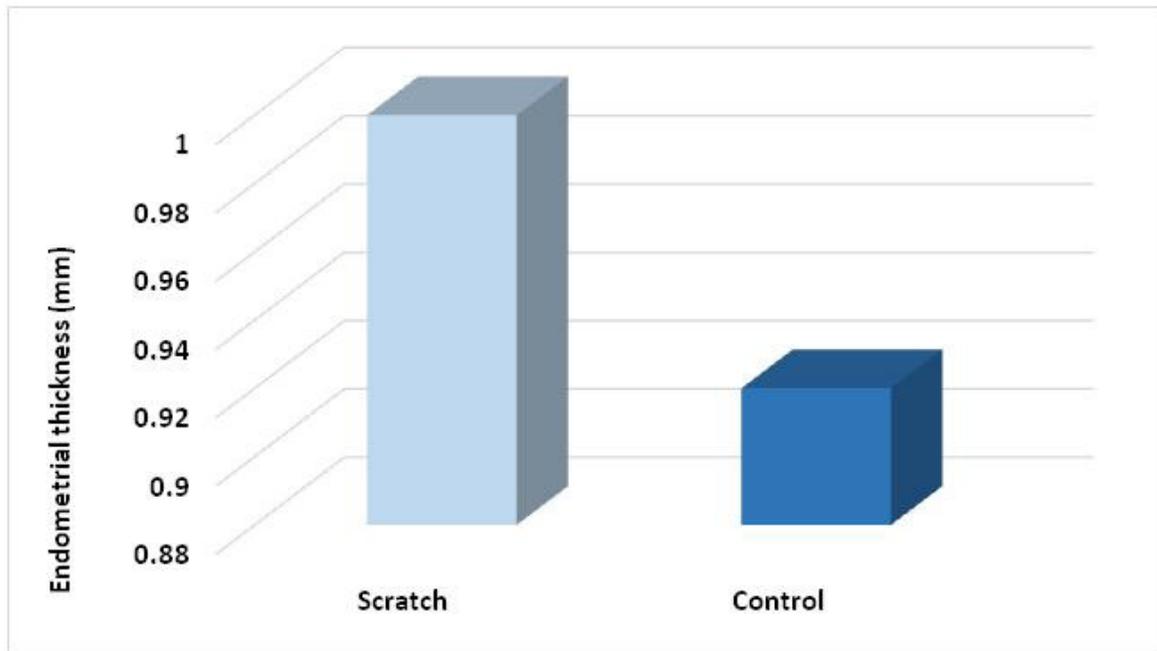


Figure 4. Comparison of endometrial thickness between scratch and control groups

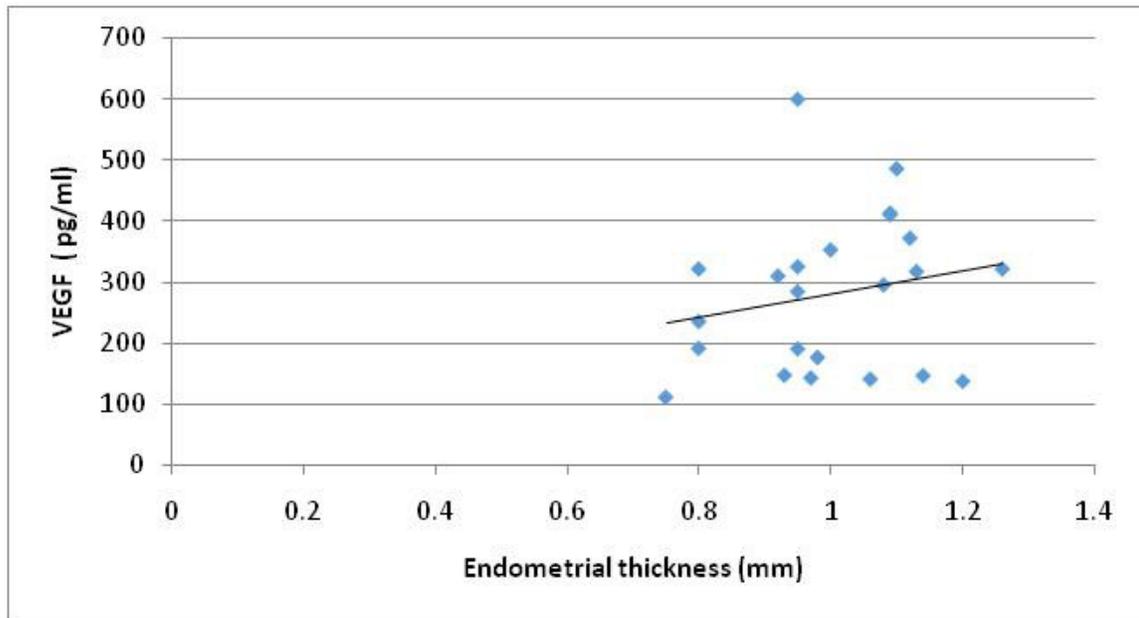


Figure 5. Correlation between endometrial thickness and VEGF in scratch group at day of embryo transfer

DISCUSSION

Despite of the unbelievable advances in ART, embryo implantation remains the limited step in the rate for the success outcome. The causes of repeated implantation failure could be multifactorial or even originate from embryonic defects (Nordqvist, 2016).

The patients in scratch group has higher pregnancy rate (14/23) 60.9% than nonintervention group (11/33) 33.3% although not significant (p value 0.057) could be due to small sample size as shown in figure 1 and this is agreed by (Gnainsky et al., 2015).

The advantageous effect of endometrial injury could be underpinned by a number of structural and functional

endometrial changes including the stromal and epithelial components of the endometrium at the molecular level. In this study the endometrial thickness of the scratch group was significantly higher than non-scratched group as in Figure 4 and this is agreed with other researcher (Wu et al., 2014). Endometrial thickness has been recognized to be a significant factor in embryo implantation in IVF cycles and by extension successful pregnancy; further evaluation of clinical pregnancy rates according to each millimeter of endometrial thickness (Chen et al., 2010).

Endometrial scratching that induces an inflammatory reaction characterized by an influx of macrophages and dendritic cells, yielding in increase local production of cytokines and growth factors, which in turn promote decidua proliferation (Junovich et al., 2011).

Also endometrial injury can stimulate epithelial cells and stromal cells to secrete VEGF. In addition, the scratching could recruit neutrophils and macrophages into endometrium (Zhang et al., 2015) and these recruited neutrophils and macrophages could secrete VEGF and IFN (Yu et al., 2015; Kasama et al., 2005).

We therefore hypothesized that endometrial injury might facilitate the expression of VEGF, thus giving a favorable environment which can enhance vascularization in maternal decidua during early pregnancy. In our study, VEGF, the regulator of angiogenesis, was upregulated after scratching, which may observe that it could increase the endometrial blood flow perfusion (Zhang et al., 2015). In addition, patients with unknown cause of infertility have higher impedance blood flow in spiral arteries compared with fertile controls, which means that impaired blood flow could be an important contributing factor to unexplained infertility. So the increasing in endometrial blood flow perfusion by VEGF upregulation may enhance endometrial thickness as we show in our study as in Figure 5. Kasama et al. (2005) revealed that VEGF levels are significantly reduced in uterine secretions during the mid-secretory phase in women with unexplained infertility compared with fertile women. And they support the concept that endometrial secretions, including VEGF, play important roles during implantation. Thus, in females with unexplained subfertility, endometrial scratch might be an important way to improve endometrial receptivity and IVF outcomes (Barash et al., 2003). In present study, there are significant differences in VEGF level P value (<0.001) in scratch group which is higher after than before the procedure. Furthermore, the VEGF level in scratch group is significantly higher than control group as p value was (0.020), as shown in fig. 2, 3. In addition to that, the endometrial thickness of the scratch group was significantly higher than non-scratched group as shown in table or fig. 4 and this is agreed with other researcher (Wu et al., 2014).

CONCLUSION

It is save and simple to use Endometrial injury induced by pipelle biopsy as an outpatient procedure which is associated with significant improvement in implantation, by providing a favorable environment which enhance endometrium and sub endometrium vascularization of the uterus. Also it improves endometrial thickness.

REFERENCES

- Alijotas-Reig J, Garrido-Gimenez C (2013). Current concepts and new trends in the diagnosis and management of recurrent miscarriage. *ObstGynecolSurv*; 68:445–466
- Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I (2003). Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing *in vitro* fertilization. *FertilSteril*; 79:1317–1322.
- Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I (2003). Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing *in vitro* fertilization. *FertilSteril*; 79:1317–1322.
- Chen S-L, Wu F-R, Luo C, Chen X, Shi X-Y (2010). Combined analysis of endometrial thickness and pattern in predicting outcome of *in vitro* fertilization and embryo transfer: a retrospective cohort study. *ReprodBiolEndocrinol*; 65: 30.
- Dekel N, Gnainsky Y, Granot I, Racicot K, Mor G (2014). The role of inflammation for a successful implantation. *Amer J ReprodImmunol*; 72:141–147.
- Gnainsky Y, Granot I, Aldo Y (2015). Biopsy-induced inflammatory conditions improve endometrial receptivity: the mechanism of action. *Reproduction*, 149:75–85.
- Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA (2004). Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev.*; 56:549-580.
- Junovich G, Mayer Y, Azpiroz A, Daher S, Iglesias A, Zylverstein C (2011). Ovarian stimulation affects the levels of regulatory endometrial NK cells and angiogenic cytokine VEGF. *Amer J ReprodImmunol*; 65:146-153.
- Kasama T, Miwa Y, Isozaki T, Oda T, Adachi M, and Kunkel SL (2005). Neutrophil-derived cytokines: potential therapeutic targets in inflammation. *CurrDrug Targets: Inflamm and Allergy*; 4:273–279, 2005.
- Kovacs P, Matyas S, Boda K, Kaali SG (2003). The effect of endometrial thickness on IVF/ICSI outcome. *HumReprod*; 18:2337–2341.
- Li R, Hao G (2009). Local injury to the endometrium: Its effect on implantation. *CurrOpinObstetrGynecol*; 21:236–239.
- Narvekar SA, Gupta N, Shetty N, Kottur A, Srinivas M, Rao KA (2010). Does local endometrial injury in the non-transfer cycle improve the IVF-ET outcome in the subsequent cycle in patients with previous unsuccessful IVF? A randomized controlled pilot study. *J HumReprod Sci.*; 3:15–19.
- Nava D, Yulia G, Irit G, and Gil M (2010). Inflammation and Implantation. *JReprodImmunol.* ; 63:17–21.
- Nordqvist C (2016). Infertility: Causes, Diagnosis, Risks, Treatments. *Med News Today*;56:254-260
- Nordqvist C (2016). Infertility: Causes, Diagnosis, Risks, Treatments. *Med News Today*;56:254-260
- Sen CK, Khanna S, Venojarvi M, Trikha P, Ellison EC, Hunt TK, Roy S (2002). Copperinduced vascular endothelial growth factor expression and wound healing. *AmerJPhysiol Heart CirculPhysiol*; 282(5):H1821-1827.
- Smith SK (2011). Vascular endothelial growth factor and the endometrium. *Hum Reprod*; 2:56–61.
- Van Der Gaast M. H., Beckers N. G., Beier-Hellwig K (2012). Ovarian stimulation for IVF and endometrial receptivity. *Reproductive*

- Biomedicine Online.; 72:710–715.
- Wu Y, GAO X, Lu X (2014). Endometrial thickness affects the outcome of *in vitro* fertilization and embryo transfer in normal responders after GnRH antagonist administration. *ReprodBiolEndocrinol.*; 12:96.
- Yu L, Junyan H, Chanwei J, Yanmin M, Yonglian L, Ying L, Shuyu W (2015). Clinical Study Effect of Endometrial Injury on Secretion of Endometrial Cytokines and IVF Outcomes in Women with Unexplained Subfertility. *Reprod TechnObstet Gynecol J Beijing/China.*;31:223-237.
- Zhang X, Chen CH, Confino E, Barnes R, Milad M, Kazer RR (2005). Increased endometrial thickness is associated with improved treatment outcome for selected patients undergoing *in vitro* fertilization-embryo transfer. *FertilSteril*;83: 336–340
- Zhang XL, Kang Y, Qi C, Zhang Q, Kuang YP (2015). Clinical observations of sequential therapy with Chinese medicine and hysteroscopic mechanical stimulation of the endometrium in infertile patients with repeated implantation failure undergoing frozen-thawed embryo transfer. *ChinJ. Integr Med*; 21:249–253.