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Evaluation of implantation and clinical pregnancy rates after endometrial scratching in women with recurrent implantation failure

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ABSTRACT

Objective: to investigate the role of endometrial scratching to improve clinical pregnancy and implantation rates in women with repeated implantation failure.

Methods: 80 patients with at least two previous failed ICSI or FIVET (failed implantation) were randomly assigned to group 1 (scratching) or group 2 (control). Endometrial scratching was performed by pipelle de Cornier® while the control group was submitted to a sham procedure using an embryo-transfer catheter. All of patients underwent to estroprogestin pill (ethinylestradiol 30 µg + levonorgestrel 150 µg) during the month before ovulation hormonal induction and IVF. Implantation and clinical pregnancy rates were compared for Mantel-Haenszel Risk Ratio test ($p < 0.05$).

Results: endometrial scratching was associated with higher clinical pregnancy (25% vs. 10%, p=0.08) and implantation (40% vs. 33%, p=0.67) rates but the comparison between the two groups was not significant.

Conclusion: endometrial scratching in the patients with repeated implantation failure during the cycle before ovarian stimulation doesn't significantly improve implantation and clinical pregnancy rates.

Keywords: scratching, in vitro fertilization, implantation, pregnancy, failure.

SOMMARIO

Obiettivo: indagare il ruolo dello scratching endometriale nell'aumentare i tassi di gravidanza clinica e di impianto nelle pazienti con multipli fallimenti dell'impianto dopo fecondazione assistita.

Materiali e metodi: 80 pazienti con almeno due precedenti ICSI o FIVET fallite (fallimento dell'impianto) sono state randomizzate e assegnate al gruppo 1 (scratching) o al gruppo 2 (controllo). Lo scratching endometriale è stato eseguito con pipelle de Cornier® mentre nel gruppo di controllo la procedura è stata simulata con un catetere da embryo-transfer. Tutte le pazienti sono state sottoposte a trattamento estroprogestinico (etinilestradiolo 30 µg + levonorgestrel 150 µg) il mese precedente alla stimolazione ovarica e alla IVF. I tassi di impianto e di gravidanza clinica sono stati confrontati con il test Mantel-Haenszel Risk Ratio ($p<0.05$).

Risultati: lo scratching endometriale è associato a tassi migliori di gravidanza clinica (25% vs. 10%, p=0.08) e di impianto (40% vs. 33%, p=0.67) ma il confronto tra i due gruppi è risultato non statisticamente significativo.

Conclusioni: lo scratching endometriale eseguito nelle pazienti con ripetuti fallimenti dell'impianto durante il mese precedente alla stimolazione ovarica non aumenta significativamente i tassi di impianto e di gravidanza clinica.

Parole chiave: scratching, fecondazione in vitro, impianto, gravidanza, fallimento.

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INTRODUCTION

In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the most frequent methods used for treating infertile couples; these techniques can be performed using fresh or frozen embryos, in which it is necessary a thawing process prior to transfer[1].

A cycle is defined as canceled when after ovarian stimulation there is not in utero transfer. A cycle can be canceled before withdrawal of oocytes (suspended cycle) or before embryo transfer (interrupted cycle). 17.354 canceled cycles were reported in Italy in 2015, representing 31,4% of all cycles with a 2,8% increase compared to 2014. 9,2% (+0,4 %) were suspended before pick-up and 22,1% (+2,3%) were interrupted before embryo transfer. From 2005 a 20% decrease of suspended cycles and a 86% increase of interrupted cycles have been registered[2].

The main reason of suspension is the absence of response to ovarian stimulation, occurring in 2/3 of canceled cycle, while 11,8% of suspensions are caused by abnormal response to ovarian stimulation. This suspension can be determined by many factors and it is directly proportional to age of patients. In fact, up to 39 years the possibility of suspension after pick-up is less than 10%, in the patients aged between 40 and 42 years the risk of suspension is 10,1% and in the patients aged more than 43 years the risk is 2,5 higher than younger patients[3].

The mean reasons of interruption are missed fertilization (22,4%) and miss oocytes' withdrawal (17,1%): both determines about 40% of interruptions. About 40000 embryo-transfer were reported in Italy in 2015 and pregnancy was obtained in 27% of cycles. The probability to get pregnant depends on multiple factors that can be summarized in two elements[4]:

- Embryonal factor: quality and quantity of transferred embryos;
- Uterine cavity: endometrial receptivity, uterine malformations and uterine disease as fibroids and polyps.

Histological and pathological changes induced by ovarian hormones during luteal phase are biomarkers of endometrial receptivity. Their expression, evaluated with endometrial biopsies, could be very useful for the study of endometrial receptivity[5].

It's been proposed to perform mechanical lesions of endometrial mucosa (biopsy/scratching or hysteroscopy) in the previous cycle or during ovarian stimulation for IVF in order to increase implantation rate in women with RIF (Repeated Implantation Failure)[6-9]. The results of systematic revision highlighted a positive effect of local lesions on endometrial mucosa in the cycle before ovarian stimulation for IVF[10]. It's been theorized that mechanical injury activates immunologic and genetic changes on endometrial mucosa with better endometrial receptivity for implantation[11,12].

The aim of this study is to evaluate the role of endometrial scratching in the patients with RIF in order to increase implantation and clinical pregnancy rates.

MATERIALS AND METHODS

It's been performed a randomized unblinded controlled trial (RCT) in a ratio of 1:1. We evaluated infertile couples to undergo in vitro fecundation at the Medically Assisted Procreation Centre of Obstetrics and Gynecologic Operative Unit, Cannizzaro Hospital (Catania, Italy). 80 patients were recruited according to following inclusion criteria:

- Age between 25 and 37 years:
- Primitive or secondary infertility;
- At least two previous failed ICSI or FIVET (failed implantation) despite easy transfer and good quality of embryos;
- Normal thickness and endometrial ultrasound pattern, defined as absence of intracavitary disease (fibroids, polyps, etc.), with no anamnestic severe deep endometriosis;
- Good quality of seminal fluid of partner and negative anamnesis for relevant diseases;
- Negative genetic, metabolic and infective evaluation.

The procedure and the objective of scratching were explained to all of patients and an informed consent was administered after institutional review board approval.

80 patients were randomly recruited according to inclusion criteria. Descriptive statistics of sample is summarized in **table 1**. All of patients underwent to estroprogestin pill (ethinylestradiol 30 μg + levonorgestrel 150 μg) during the month before ovulation hormonal induction and IVF.

Gynecological examination and transvaginal ultrasound were performed between the 14th and 16th day of pill in order to evaluate size and morphology of uterus, ovaries and to start consequently administration of GnRH agonist (buserelin).

Table 1.
*Descriptive statistics of women undergoing assisted reproduction allocated to endometrial scratching (n=40) or sham procedure (n=40). *Chi-square test. ** Kruskal-Wallis test. CC, clomiphene citrate; FSHr, recombinant follicle stimulating hormone; hMG, human menopausal gonadotropin*

PARAMETERS	ENDOMETRIAL SCRATCHING	SHAM PROCEDURE	P
AGE (MEDIAN)	32	31	** 0.33
PREVIOUS FAILED ICSI OR FIVET	30/40 (75%)	31/40 (77,5%)	* 0.79
2			
3	10/40 (25%)	9/40 (22,5%)	
OVARIAN STIMULATION PROTOCOL	11/40 (27,5)	10/40 (25%)	* 0.95
• CC+hMG + Antagonist			
• FSHr + Agonist	14/40 (35%)	15/40 (37,5%)	
• FSHr + Antagonist	15/40 (37,5%)	15/40 (37,5%)	
N. TRASFERRED EMBRYOS	9/40 (22,5%)	3/40 (7,5%)	* 0.68
1			
2	16/40 (40%)	8/40 (20%)	
≥3	5/40 (12,5%)	1/40 (2,5%)	

Patients were randomly assigned to group 1 (scratching) or group 2 (control). Endometrial biopsy by pipelle de Cornier® (Laboratoires PRODIMED, Neully-EnThelle, France) were performed in the patients of group 1. After disinfection of vagina and portio with iodate solution, the pipelle was introduced gently through the cervix up to the uterine fundus. The piston was then drawn back to the end of the biopsy cannula until it self-locked, creating a negative pressure. Aiming to cover the entire endometrium, the examiner applied regular first back-and-forth movements (3–4 cm) and then rotating the sampler, over the whole uterine cavity, during a period of 30 s. The entire procedure duration was up to 5 minutes. Endometrial scratching was performed by a dedicated team (2 operators only) during luteal period, between 5 and 10 days before menstruation.

The control group was submitted to a sham procedure using an embryo-transfer catheter introduced along the cervix inside the uterine cavity. A visual analog scale consisted of an horizontal line between 0 (no pain) and 10 cm (worst pain) was administered to all of patients. Each patient marked a point on this scale representing the pain related to procedure.

A GnRH analogue was administered together with estroprogestin pill until to HCG intake. Hormonal and sonographic evaluation was performed after 15 days from starting administration of gonadotropins (rFSH and/or HMG) with tailored dose for each patient.

The monitoring consisted of sonographic and hormonal (17- β estradiol, prolactin and LH) control at day 5 after gonadotropins intake and then every other day. The entire gonadotropin therapy duration was between 11 and 12 days. Human Chorionic Gonadotropin (HCG) was used in order to stimulate oocyte's growth with a dose of 5.000-10.000 UI after sonographic evaluation of endometrial thickness (range 8-12,5 mm); oocyte's pick-up was performed after 34-36 hours.

Adequate number of oocytes was obtained from pick-up procedures, without complication. 2 or 3 embryos were transferred in utero 48-72 hours after oocyte withdrawal. Only one case of uterine mucocele 2 day after pick-up was registered, so the embryo-transfer was delayed to the next day, after aspiration of mucocele and evidence of regular sonographic endometrial pattern.

Luteal period support therapy was performed by administration of intramuscular progesterone 50-100 mg/die and vaginal estradiol valerate 2-6 mg/die, up to negative pregnancy test or 12 weeks of gestation. Serum β-hCG was measured out 14 days after embryo-transfer.

Clinical pregnancy is defined as ultrasound evidence of gestational sac with beating heart embryo[13].

We calculated implantation and clinical pregnancy rates and then statistical analysis by SPSS and Excel software for Mantel-Haenszel Risk Ratio test. 0.05 was used as the cutoff value for probability significance ($p < 0.05$).

RESULTS

All of recruited patients completed reproductive procedures including embryo-transfer. 12 of 40 patients of group 1 (endometrial scratching) were positive to β HCG dosage performed 14 days after embryo-transfer; serum β HCG was measured weekly until to 4-5 weeks after embryo-transfer. Then transvaginal sonography was performed. 10 of 12 patients were pregnant: intrauterine gestational sac, yolk sac, vital embryo with regular crown-rump length were detected. Clinical pregnancy rate was 25% (10 pregnancies of 40 patients). 8 pregnancies were singleton and 2 twin gestations. Overall 30 embryos were transferred resulting in 40% implantation rate (12/30) while cumulative implantation rate was 11% (12/108).

In the group 2 (control) 6 of 40 patients were β HCG positive while only 4 of them

had sonographic evidence of pregnancy (vital embryo), so the clinical pregnancy rate was 10% (4 pregnancies/40 patients). Overall 12 embryos were transferred resulting in 33% (4/12) implantation rate while cumulative implantation was 3,5% (4/112).

The comparison of clinical pregnancy and implantation rate between group 1 and 2 was not significant (**table 2**).

Table 2.
Mantel-Haenszel Risk ratio

	Group 1 (scratching)	Group 2 (control)	RR (CI 95%)	P
Clinical pregnancy rate	10/40 (25%)	4/40 (10%)	**0.83 (0.67-1.02)**	0.08
Implantation rate	12/30 (40%)	4/12 (33%)	**0.90 (0.54-1.4)**	0.67

30 of 40 patients declared a pain from 5 to 7, 6 patients a value of 2 and 4 patients a value of 8, so the scratching should be a procedure whit moderate pain. In the control group the median pain was inferior to 5.

Very low bleeding (<50 ml) was registered only during the scratching in the major part of group 1 patients (31/40, 77%).

DISCUSSION

Correlation between endometrial quality in proliferative phase (mostly before the transfer) and probability to get pregnancy was evaluated in the past years since the 90s and sonographic study of endometrium was introduced as uterine receptivity marker[14,15]. Particularly thickness and pattern are the parameters which have to be studied in order to obtain required information[16]. Many publications support at least 7 mm thickness to have good probability of implantation and 2,5 ml is the cutoff volume to get pregnancy established from researches using tridimensional ultrasound[17]. Endometrial pattern evaluation is based on endometrial and myometrial echogenicity in longitudinal section. A "low grade" pattern is correlated with low success rate; it is displayed as homogeneously hyperechogenic without echogenic middle line. Conversely trilaminar pattern is associated with good endometrial receptivity and better conception rates[18].

The Doppler study of uterus vascularization in unfertile women revealed that lower perfusion of uterine and spiral arteries with absent endometrial and sub-endometrial flow causes low probability of implantation[19].

However, many studies investigated other markers of endometrial receptivity as cytokines, interleukins and hormones. High progesterone level before oocyte withdrawal change genetic profile of endometrium during implantation time and molecular way majorly influenced is the cytotoxic way by Natural Killer cells, in which about 16 genes are altered. Particularly 5 of these encode for receptors that are overexpressed on trophoblastic fetal cells when progesterone level is higher, resulting in failing implantation.

It has been demonstrated also that Natural Killer cells levels are positively correlated with some angiogenic steps; overexpression determines oxidative stress and altered placental circulation. Other proteins like cytokines, growth factors, integrins, glycodelin and LIF (Leukemia Inhibitory Factor) have been recognized as predictive of endometrial receptivity, but they are not useful for current clinical activity[20].

Furthermore it's known a positive correlation between pinopodes expression and pregnancy rates in the patients undergone to hormone replacement therapy and egg donation, and also a correlation between lower expression of pinopodes and repeated implantation failures. Studies on endometrial biopsies highlighted higher concentration of pinopodes at 2 cm from uterine fundus, identifying it as preferential situs of implantation[21]. For the major part of infertile couples limiting factor is the implantation, by also little known causes. The term RIF, Recurrent Implantation Failure, used from 1983, represent the absence of implantation or clinical pregnancy after repeated embryo-transfer.

Endometrial receptivity is one of key factors regulating blastocyst implantation and it has been demonstrated that mechanical trauma on endometrial mucosa determines inflammatory response with release of cytokines and growth factors like IL-6 and TNF-α, making endometrium more prepared for implantation[22]. Furthermore it determines genic expression modulation with higher expression of favorable protein for implantation, like MUC1 (mucin 1 transmembrane), crystalline alpha B, APOD (apolipoprotein D), PLA2 (phospholipase A2) and UPIb (uroplakin Ib), glycodelin A, laminin alfa 4, integrin alfa 6 and MMP-1 (metalloproteinases 1), and recruitment of macrophages and dendritic cells playing an important role on decidualization and implantation[23].

This effect is maintained also in the next cycle,

probably because recruited monocytes live and stay longer in the site of interest. In order to improve results in women with RIF, many study have been take in consideration pregnancy rates after local trauma on endometrium in the cycle before ovarian stimulation, including only patients with regular uterine cavity at hysterosalpingography or hysteroscopy. All of these studies showed high clinical pregnancy rates in the patients undergone to hysteroscopy[24-26]. Number of sampling among these studies is different: one time[27], one sampling between 7 and 10 days and then another one 24-25 days after previous cycle[28], 4 times (days 8, 12, 21, 26) in the cycle before ovarian stimulation[29].

Successful of implantation is a complex process that needs a receptive endometrium, good embryos at blastocyst state and synchronization between maternal and embryonal tissues. In order to improve implantation rate, current evidence is that patients should have ready frozen embryos to be successively transferred during a natural cycle. Alternatively endometrial scratching could be performed in the cycle before ovarian stimulation. Number, time and modality of scratching have to be still defined.

In our study clinical pregnancy and implantation rates did not differ significantly between the examined groups. In the literature concerning the role of endometrial scratching to improve clinical pregnancy and implantation rates, there are three not-randomized[26,29,30], and five randomized studies[8,9,28,31,32], demonstrating benefit of this technique.

Our research differs from the other studies for the inclusion criteria, taking into consideration only patients with two or more previous failure. Our results can be compared only with Nastri study of 2013[7], which examined 158 women underwent to estro-progestin treatment and successive endometrial scratching, demonstrating significant improve of clinical pregnancy and birth rates but worse pain score. Despite the limits of our study including poor number of patients and absence of sonographic parameters (endometrial thickness, endometrial volume, uterine Doppler velocimetry), our preliminary data should demonstrate that endometrial scratching in the patients with RIF during the cycle before ovarian stimulation doesn't significantly improve implantation and clinical pregnancy rates.

Currently many questions are still open on endometrial scratching. The precise subgroup of patients majorly need of scratching (for example the patient with RIF) has to be identified. The procedure could increase the risk of latent infections and consequently it could determine sub-fertility. The best approach for scratching (hysteroscopy versus endometrial biopsy) has to be investigated. Further studies are necessaries to resolve these unanswered questions.

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