

Effect of local endometrial injury in Follicular vs. luteal phase on implantation and pregnancy outcomes of Frozen Embryo Transfer Cycles in women with repeated in vitro fertilization failure. A randomized clinical trial

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Research Article

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Abstract

Introduction

:recurrent implantation failure(RIF)refers to failure to achieve a clinical pregnancy after transferring at least four good quality embryos in a minimum of three fresh or frozen cycles.Endometrial scratch is the newly proposed surgical strategy to improve endometrial receptivity;the healing process following scratch may release cytokines and growth factors which encourage endometrial growth and facilitates implantation.Timing of this procedure is a point that may lead to different outcomes.This study compares follicular versus luteal phase injury due to their different molecular patterns on the outcome of embryo transfer in RIF patients.

Study design:

This study is a double blinded randomized clinical trial.Patients came into infertility clinic of Arash women's hospital in years2017and2018are divided into two groups. In one group injury was done with pipelle at4th-6thday of frozen embryo transferring cycle(n = 38)and in the other one it was done at21th-23thday of the cycle before the FET (n = 40).Then the infertility rates were compared.

Results

Totally29(41.4%)chemical pregnancies happened,15(51.7%)of them was in follicular group and14(48.3%)in luteal group.28(40%)clinical pregnancies happened totally which were equal in both groups(14 in each one).Ongoing pregnancies happened in 22(31.4%)patients that12(54.5%)of them were in follicular and the other10(45.5%)were in luteal group.

Conclusion

According to this study,local endometrial injury by pipelle didn't show any significant differences in both groups pregnancy outcomes.But in the follicular group the IVF cycle time period is decreased and therefore the psychological burden of long time required for luteal phase LEI will decrease and is more acceptable for patients,maybe it's better to do LEI in follicular phase;more studies with larger sample sizes recommended.

Introduction:

Infertility defined as one year of unprotected intercourse without conception [2–3]. Major developments have occurred in recent years, in vitro fertilization is one of them that could be used in almost all infertility cases.

IVF success rate is about 30 to 40 percent in each cycle [4]. It depends on factors like woman's age, infertility cause, treatment protocol, embryo quality, number of transmitted fetuses, transmission technique, sperm quality and endometrial characteristics [5].

Repeated implantation failure (RIF) is failure of implantation of transferred

embryos after several IVF treatment cycles. However, there are no formal criteria defining the number of failed cycles or the total number of embryos transferred in these IVF attempts so, there are various definitions in different infertility centers. usually RIF defines as failure of implantation in at least two or three consecutive IVF attempts, in which 1–2 embryos of high grade quality are transferred in each cycle. [6]. These failures can cause psychological and financial burden to the patients and community and causes wasting times. Therefor many researchers focus on finding ways to increase the implantation rates.

Environmental and fetomaternal factors affect pregnancy outcomes. Endometrial cavity abnormalities, inappropriate endometrium, thrombophilias, connective tissue disorders and immunological disorders are examples of the maternal factors that affect pregnancy and genetic disorders are of fetal factors [7–8–9]. Embryo quality and receptive endometrium condition are two important factors for successful implantation (10). If the endometrium is not suitable for implantation, regarding the quality of embryo, implantation does not occur. Local endometrial injury could evoke local inflammatory responses by rising in cytokines and growth factors and causing expression of genes that contribute in fetus implantation so it can cause higher pregnancy rate in women with RIF [11–12–13].

Many studies reported on contradictory results of endometrial injury in women with RIF. Some studies showed that implantation rates, and clinical pregnancy rates improved by LEI [14–18], and some of them showed no differences [19–20].

Juan et al. studied a systematic review about the Impact of local endometrial injury on in vitro fertilization/intracytoplasmic sperm injection outcomes and found conflicting data [21].

Studies regarding this issue are conflicting results. Endometrial injury in most of studies was performed in either follicular phase or luteal phase, or in both phases.

The effects of endometrial injury in follicular vs. luteal phase have been studied only in one study previously. Furthermore, timing of this procedure is a point that may lead to different outcomes (30). This study compares follicular endometrial injury to luteal phase injury due to their different molecular patterns on the outcome of embryo transfer in RIF patients.

Because of the long time required in luteal phase local endometrial injury of FET cycles and its consecutive psychological and financial costs, it seems that doing LEI in follicular phase would be helpful for these discomforts rather than luteal phase. This study is going to compare the effect of LEI in follicular phase vs. luteal phase of frozen embryo transferring cycles of IVF in women with RIF.

Methods:

Study design and participants:

This study is a double blinded randomized clinical trial. This study is designed as a clinical trial with a parallel random allocation and a 1: 1 ratio.

A total of 78 patients of infertility clinic of Arash women's hospital with at least one IVF failure in years 2017 and 2018 were included in this study. The inclusion criteria were: (i) infertile women with at least one IVF failure; (ii) ≤ 40 years of age; (iii) a normal uterine cavity demonstrated by hysteroscopy or

hysterosalpingography done during one year ago; (iiii) not using OCP or GnRh

agonists in the previous cycle.

The exclusion Criteria were: (i) abnormal uterine cavity like polyp, fibroid or Asherman syndrome; (ii) Endometrial hyperplasia or endometrial cancer; (iii) active vaginal or cervical infections (iiii) systemic disease such as diabetes mellitus, hypertension, and systemic lupus erythematosus or etc. Recruited participants were equally randomized into two groups using a table of block randomization: follicular group (group 1: endometrial injury in proliferative phase, n = 38); and luteal group (group 2: injury in luteal phase, n = 40).

This study was approved by the Institutional ethics committee Review Board of Tehran medical university (IR.TUMS.MEDICINE.REC.1396.2911) all participating patients recruited to the study were fully counseled and signed written informed consent.

Techniques:

Patients of follicular group were treated with estradiol valerate for 10 to 14 days and simultaneously the endometrial injury was performed by biopsy catheter (Pipelle; de Cornier, Prodimed, Neuilly-en-Thelle, France) in days 4–6 (if the bleeding stopped) of menstrual cycle before transmitting frozen embryos in uterus. This procedure performed as follows: the Pipelle was inserted through the cervical os and advanced gently until resistance was felt and after scratching, it was brought out of uterus.

After 14 days, transvaginal sonography was done and if the triple line endometrium was seen or the echogenic endometrium was ≥ 7 mm, progesterone suppository was prescribed and after 3–4 days, 2 or 3 days frozen

fetuses were transmitted in uterus. After 2 weeks serum beta hcg was checked

and if it was more than 15 milli International Units per milliliter, trans vaginal sonography was performed for finding gestational sac(s). If it was seen, pregnant women were followed up to 12 weeks.

In group 2, the same procedure was done but the endometrial injury with pipelle was performed in days of 21–23 of the menstrual cycle before FET.

The outcomes were described as (a) chemical pregnancy: positive serum beta hcg, (b) clinical pregnancy: existence of gestational sac(s), (c) implantation rate: the proportion of gestational sacs to transferred fetuses, and (d) ongoing pregnancy: being pregnant after 12 weeks.

Limitations:

Long period of following patients was a limitation of the study which was resolved by calling patients and free visits.

Data analysis:

Stata ver.13 was the analyzing software. The baseline characteristics of the two groups of patients were compared by chi-square and independent T-test. For determining the relationships between the injury time and pregnancy outcomes logistic regression was used. P-value was 5 percent.

Results:

A total of 78 patients were recruited in this study from 2016 to 2017, 38 patients were in follicular group which pipelle was performed on days 4 to 6 of the FET cycle and 40 patients were in luteal group which pipelle was performed on days 21–23 of the cycle before FET. It was 8 patients' loss during follow up, 3 from follicular group and 5 from luteal group. They were unavailable to see their outcomes. Therefore, there were 35 participants in each group finally.

The average age was 31.37 years in follicular and 31.2 years in luteal group. The average years of infertility and body mass index(BMI) in both groups were about the same. Also the number of retrieved and freezed ovocytes and transferred eggs were compared and there were no significant differences. Mean endometrial thickness at the time of transferring was 9.21 mm in follicular and 9.67 mm in luteal group which they didn't differ statistically.

Both groups were compared from the aspect of infertility causes. Male infertility rate accounts for 51.4% of infertility causes overall which was 47.2% in follicular and 52.8% in luteal group. Unexplained infertility was another cause of infertility (21.4% generally) that was happened 53.3% in follicular and 46.6% in luteal group. Other causes of infertility were PCOD, tubal factor and endometriosis which account for 34.2%, 8.5% and 4.2% of all contributors respectively. Statistically, no differences were seen.

Next, infertility types evaluated. Generally primary infertility rate was 78.5% and secondary type was 21.4%. they are shown in the table.

In Table 1 basic characteristic of both groups are shown and there is not statistical differences.

Table 1
basic characteristics of patients undergoing endometrial local injury in
follicular and luteal phases

Variables	Follicular group	Luteal group	P value
Age(y)	31.37 ± 5.35	31.2 ± 5.51	0.912
BMI(Kg/m ²)	24.64 ± 2.69	25.65 ± 3.01	0.387
Duration of sub fertility	6.6 ± 4.55	6.02 ± 4.46	0.942
Oocyte number	17.68 ± 7.91	19.48 ± 5.38	0.24
frozen embryo number	6.17 ± 3.36	6.48 ± 2.46	0.22
Transferred embryo number	2.65 ± 0.68	3.05 ± 0.9	0.489
Endometrial thickness	9.21 ± 1.42	9.67 ± 1.74	0.251
Types of infertility			0.38
Primary	26(74.3%) ¹	29(82.85%)	
secondary	9(25.7%)	6(17.14%)	
Causes of infertility			0.632
Male factor	17(48.57%)	19(54.28%)	
Tubal factor	4(11.42%)	2(5.71%)	
PCO	11(31.42%)	13(37.14%)	
Endometriosis	3(8.57%)	0	
Unexplained	8(22.85%)	7(20%)	
1 = percent of the participants in their groups.			

Finally, both groups evaluated for pregnancy outcomes. 29 cases of successful Chemical pregnancy happened (41.4%) that was 51.7% in follicular and 48.3% in luteal group. In clinical pregnancy, 28 cases were successful, which divided equally in both groups. 22 cases of ongoing pregnancy happened that its percentage was 54.5 for follicular and 45.5 for luteal group. Abortion occurred in 10% of participants. 57.1% in follicular and 42.8% in luteal group were seen. Implantation rate generally was 0.19 with SD = 0.28. its rate in follicular group was 0.21 (SD = 0.28) and in luteal group was 0.17 (SD = 0.27). Statistically no significant differences were seen in pregnancy outcomes.

In Table 2 the pregnancy outcomes are shown and no significant differences were seen.

Table 2
Pregnancy outcomes of patients undergoing endometrial injury in proliferative phase and luteal phase (percent in each group)

Outcomes	Follicular group	Luteal group	P value
Chemical pregnancy	15(42.85%)	14(40%)	0.808
Clinical pregnancy	14(40%)	14(40%)	1
Ongoing pregnancy	12(34.28%)	10(28.57%)	0.607
Abortion rate	4(11.42%)	3(8.57%)	0.643
Implantation rate	0.21 ± 0.28	0.17 ± 0.27	0.5

In Tables 3, 4 and 5 the demographic and IVF factors effects on chemical, clinical and ongoing pregnancy is shown.

Table 3
demographic and IVF factors effects on chemical pregnancy

Factors	Successful chemical pregnancy	Unsuccessful chemical pregnancy	P-value
Mean age	31 ± 5.41	31.48 ± 5.44	0.303
Male infertility	15(51.72%)	21(51.21%)	0.967
Female infertility	21(72.41%)	27(65.85%)	0.075
embryo grade	A = 21 B = 0 AB = 8	A = 27 B = 2 AB = 12	0.463
BMI	24.99 ± 3.1	25.25 ± 2.7	0.706
Endometrial thickness	9.5 ± 1.6	9.39 ± 1.5	0.435
Fetus transferred	2.75 ± 0.6	2.92 ± 0.9	0.403

Table 4
demographic and IVF factors effect on clinical pregnancy

Factors	Successful clinical pregnancy	Unsuccessful clinical pregnancy	P-value
Mean age	30.82 ± 5.43	31.59 ± 5.42	0.332
Male infertility	15(53.57%)	21(50%)	0.770
Female infertility	20(71.42%)	28(66.66%)	0.099
embryo grade	A = 20 B = 0 AB = 8	A = 28 B = 2AB = 12	0.499
BMI	24.89 ± 3.1	25.31 ± 2.6	0.56
Endometrial thickness	9.51 ± 1.6	9.38 ± 1.5	0.78
Fetus transferred	2.82 ± 0.6	2.88 ± 0.9	0.76

Table 5
demographic and IVF factors effect on ongoing pregnancy

Factors	Successful ongoing pregnancy	Unsuccessful ongoing pregnancy	P-value
Mean age	30.72 ± 5.02	31.54 ± 5.59	0.506
Male infertility	11(50%)	25(52.08%)	0.871
Female infertility	16(72.72%)	32(66.6%)	0.249
embryo grade	A = 16 B = 0 AB = 6	A = 32 B = 2AB = 14	0.602
BMI	3.03 ± 24.77	2.83 ± 25.31	0.46
Endometrial thickness	1.7 ± 9.65	1.5 ± 9.33	0.43
embryo transferred	0.63 ± 2.86	0.89 ± 2.85	0.96

None of them were significant.

Discussion:

In this study, we try to assess whether there is a difference between follicular phase and luteal phase endometrial injury on pregnancy outcomes. In our study there was no difference between injury in the follicular phase or luteal phase on pregnancy and implantation outcomes.

There are multiple factors that affect implantation of the embryo other than oocyte or sperm quality, and endometrial receptivity is one of them which is important [21–22].

Kalma et al. reported that endometrial biopsies cause the rise in membranous proteins expression like Uroplakin 1b. Some believe that endometrial injury before embryo transfer could cause decidualization and improves Implantation. Also Inflammatory response and rise of cytokines, interleukins, growth factors, dendritic cells and macrophages could improve plantation. Endometrial injury could improve synchronicity of embryo and uterus [24–25].

The effect of endometrial injury on improving pregnancy outcomes has been studied previously and there are many conflicting results.

Barash et al. showed that repeated endometrial biopsies by a Pipelle catheter on days 8, 12, 21, and 26 of the spontaneous menstrual cycle before the IVF-ET treatment doubled clinical pregnancy and live birth rates in women with RIF. They used fresh embryos but in our study there were frozen embryos which could decrease the implantation rate by itself [14].

Raziel et al. demonstrated that endometrial injury by pipelle catheter on days 21 and 26 of luteal cycle of women with RIF improved pregnancy outcomes significantly. They used pipelle catheter to obtain multiple biopsies of endometrium in each participant but we used it just to scratch the endometrium and didn't obtain biopsies[26].

Karimzadeh et al. on 2009 reported significantly improved implantation and clinical pregnancy in the group of women with RIF who underwent endometrial biopsy by pipelle in luteal phase of fresh cycles[15].

Narvekar et al. performed endometrial biopsy by pipelle catheter and hysteroscopy on 7th to 10th day of the cycle prior to the fresh embryo transfer cycle and once again between 24th to 25th day of the nontransfer cycle only by pipelle in women with RIF, and they showed that the live birth among this group was significantly higher than the control group [27].

Nastri et al. reported that the live birth and clinical pregnancy in the patients who endometrial injury was performed in follicular phase(on days 7 to 14 of menstrual cycle before fresh embryo transfer) by pipelle catheter were significantly improved, they used oral contraceptive pretreatment before the IVF cycle [28].

Relji et al. performed LEI in the luteal phase of the cycle preceding ovarian stimulation in an office hysteroscopy setting. Multiple endometrial injuries approximately 2 mm in depth and width in the upper part of the uterine cavity were performed using grasping forceps or scissors. They demonstrated that it can improve implantation and pregnancy outcomes [29].

In a recent study Wenjie et al. compared LEI in luteal phase preceding IVF with follicular phase. For patients in proliferative phase group, endometrial injury was performed between cycle day10–12. For patients in luteal phase group, endometrial injury was performed 7–9 days after ovulation. The endometrial injury procedure was performed in a standard approach using a Pipelle catheter (Shanghai Jiabao Medical Healthy Science Company, Shanghai, China). The Pipelle catheter was introduced through the cervix up to the uterine fundus. The piston was drawn back to the end of the sheath to create

a negative pressure. The sheath was rotated and moved back and forth within the uterine cavity to ensure adequate endometrial tissue has been obtained. Those patients in control groups had the Pipelle catheter inserted through the cervix but no injury was performed to the endometrium. They reported that there are no significant differences in implantation, pregnancy and live birth rates [30].

But Melnick et al. recently showed that LEI with the pipelle catheter in luteal phase did not increase implantation and clinical pregnancy rates. endometrial biopsies were performed with the use of a Pipelle endometrial suction curette (Cooper Surgical) 5–12 days after detection of an LH surge and they used fresh embryos [20].

The local endometrial injury can be induced by endometrial biopsy (scratch) or hysteroscopy, and it has been shown that endometrial biopsy is twice as effective as opposed to hysteroscopy [31].

We used pipelle catheter to make endometrial scratch because it was inexpensive and having minimal side effects, also it was more acceptable by our patients.

These conflicting data probably are because of different phases of endometrial injury, different cycles, different devices used for injury, various numbers of biopsies and many other factors.

Previous studies focus on injuries in luteal or follicular phase or both but by the best of our knowledge there are limited studies compare these two phases.

Conclusion:

There are no significant differences in implantation and pregnancy rates between follicular and luteal phase local endometrial injury with the pipelle catheter. Studies with larger sample sizes are needed.

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