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Is the length of time between endometrial scratching and embryo transfer important for pregnancy success? An observational study

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SUMMARY

OBJECTIVE: This study sought to evaluate the influence of time (early <90 days and late >90 days) and endometrial injury on pregnancy success.

METHODS: This is a retrospective study in which all infertile women who underwent at least one in vitro fertilization cycle at Clinica Gera between 2010 and 2015 were considered for inclusion. We included patients with a normal ovarian reserve and regular menses at intervals of up to 30 days. A total of 315 patient files were reviewed, and the study group was composed of patients who faced fertility issues and had male-caused infertility or idiopathic infertility. Also, women with male or unknown cause of infertility who have performed endometrial biopsy and have undergone embryo transfer up to 180 days after this procedure between 2010 and 2015 were included. The patients were divided into two groups according to the interval between biopsy and embryo transfer: group 1 (early—an interval of <90 days) and group 2 (late—an interval of >90 days and up to 180 days).

RESULTS: The results were superior for the group with an interval of less than 90 days relative to the group with an interval of more than 90 days ($p<0.04$). The pregnancy rates for group 1 and group 2 were 58.5% and 43.4%, respectively. The odds ratio for pregnancy success was 1.63 (95% confidence interval: 1.04 to 2.55).

CONCLUSION: The early transfer of embryos (<90 days) may produce better results with a high rate of pregnancy. Further studies are necessary to identify the mechanism involved in this phenomenon.

KEYWORDS: Endometrial cycle. Embryo transfer. Pregnancy rate. Pregnancy outcome. In vitro fertilization.

INTRODUCTION

Embryo implantation is a process that involves the apposition and adhesion of a blastocyst to the endometrium, followed by trophoblast invasion into endometrial epithelial cells. Such events occur in a receptive endometrium that has been stimulated by the ovarian steroids estrogen and progesterone[1]. Embryo implantation, which is an important requirement for a successful pregnancy, can only occur in a receptive uterus. In humans, the uterus becomes favorable to embryo implantation between days 19 and 23 of the menstrual cycle, a period known as the implantation window[2]. At present, implantation is the critical step that limits the success of in vitro fertilization (IVF) techniques[3].

In 2003, Ejzenberg et al.[4] explored the possibility that local injury to the endometrium may increase implantation rates and, therefore, improve pregnancy success. A total of 134 "good responder" patients were studied, 45 of whom underwent repeated endometrial biopsies before undergoing an IVF cycle. The pregnancy rate was approximately two times higher in the endometrial biopsy group than that in the control group, indicating that local damage induced by the biopsy may have beneficially affected the outcome of the IVF cycle; however, the mechanisms involved in this effect are unclear[4].

Narvekar et al.[5] suggested that biopsy during the cycle preceding an IVF cycle was more effective than biopsy during the conventional fertilization cycle. In addition, Gnainsky et al.[6] suggested that endometrial biopsy may promote inflammatory responses that attract pro-inflammatory cytokines, which are important to the implantation process. In particular, these substances cause the endometrial epithelium to produce molecules that favor interactions with blastocyst apposition and improve adhesion to the uterine wall[2]. This phenomenon may partially explain the effect of endometrial injury on pregnancy success. However, this effect may be temporary. Therefore, the delay on the embryo transfer might influence the results of pregnancy outcome.

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In investigations after an unsuccessful IVF cycle, for cases involving a good-quality embryo, evaluation of the endometrium[7] is performed prior to the subsequent embryo transfer, which is dependent on the endometrial biopsy results, laboratory conditions, and patients' desires. Embryos remain cryopreserved while scheduling is determined, and the duration of the interval between endometrial biopsy and embryo transfer may vary greatly in some cases. Consequently, the frozen and thaw process might influence the oocyte quality. However, the data of the length between endometrial injury and embryo transfer may affect the pregnancy rate. The aim of this study was to assess whether the length of frozen embryo may interfere with pregnancy success in assisted human reproduction after endometrial injury.

METHODS

Study design and setting

The study was retrospective observational. All infertile women who underwent at least one cycle at Clinica Gera located in the city of São Paulo, Brazil, between 2010 and 2015 were considered for inclusion. In addition, the Disciplina de Ginecologia do Departamento de Obstetrícia e Ginecologia of the Faculdade de Medicina da Universidade de São Paulo analyzed study data and validated the data of medical chart. A total of 455 patient files were initially reviewed, and the study group was composed of patients who faced fertility issues and had male-caused infertility or idiopathic infertility. The research ethics committee of IRB of Medical School USP approved this study (number 070/14, dated: April 2, 2014).

Participants

Eligibility criteria

Women with regular menses at intervals of up to 30 days with male or unknown cause of infertility who have performed endometrial biopsy and have undergone embryo transfer up to 180 days after this procedure between 2010 and 2015 were included. We excluded patients with an abnormal ovarian reserve (follicle-stimulating hormone [FSH] >12, estradiol >80 pg/mL, and less than eight antral follicles throughout the 3-day cycle of pelvic ultrasound exposure), diabetes mellitus, systemic arterial hypertension, ovarian failure, the chronic use of any medicine, any type of endocrinopathy, rheumatologic disease, chronic anovulation, or other conditions that may interfere with the endometrium, such as a sexually transmitted disease. After biopsy, the women with endometritis or functional micropolyps were excluded.

Procedures (data and sources)

All patients underwent a physical examination and routine laboratory tests to exclude female causes of infertility after failure of an IVF cycle or ovarian stimulation. Endometrial biopsy was conducted in a superior-to-inferior direction; a silicone urethral catheter (#8) coupled to a 10-mL syringe was used to create a vacuum in the entire endometrial cavity. Prior to the performance of any endometrial biopsies or sampling, a diagnostic hysteroscopy was used to examine the patient's uterine cavity, with saline solution as the distension medium and no anesthesia. Patients with endometrial polyps, submucosal myomas, or synechiae revealed by hysteroscopy were not included in the final analysis.

Samples were fixed with 4% formaldehyde in Tris-buffered saline for 24 h and then dehydrated with serially increasing concentrations of graded ethyl alcohol (EtOH) (30, 50, 70, 80, and 90%). Subsequently, they were diluted in TBS and finally in 100% EtOH. EtOH was replaced with isopropyl alcohol before samples were embedded in paraffin wax and mounted. The paraffin block was cut into thin, 5-µm-thick sections using a sledge microtome (Leica Microsystems, Wetzlar, Germany)[7]. Two independent pathologists received only histological sections of the endometrium and were blinded to patient information. Patients with endometritis or functional micropolyps were not included.

Embryo quality

Good-quality embryos with 4 to 8 cells, morulae, or blastocysts were washed twice in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered HTF medium supplemented with 10% SSS. These embryos were placed in an equilibration solution (1 mL) containing 20% (v/v) ethylene glycol (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 24% (w/v) Ficoll 70 (Pharmacia Biotech, Uppsala, Sweden), and 0.4 mol/L trehalose (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) for approximately 3 min at room temperature (25°C) under a dissecting microscope. After equilibration, embryos were placed into a vitrification solution (1 mL) containing 40% (v/v) ethylene glycol, 18% (w/v) Ficoll 70, and 0.3 mol/L trehalose for30 s at room temperature (25°C). Embryos were then placed into a 0.25 mL plastic straw (IMVTechnologies, L'Aigle, Basse-Normandie, France) that was loaded with the vitrification solution using a fine pipette, and the end of the

straw was heat sealed. The straw was positioned vertically in liquid nitrogen vapor for 30 s and was then plunged into the liquid nitrogen.

The straw was taken out of the liquid nitrogen, remained in air for 10 s, and was then immersed into a 37°C water bath for 10 s. After the sealed end of the straw was cut off, embryos were expelled into a warming solution composed of HEPES-buffered HTF medium (1 mL) containing 5% SSS and 1.0 mol/L trehalose. The embryos were kept on a heated plate at 37°C for approximately 5 min under a dissecting microscope. The cryoprotectant was removed by placing embryos for 2 min each in serial dilutions of trehalose (0.5, 0.25,0.125, 0.0625, and 0 M) in HEPES-buffered HTF medium containing 5% SSS that were on a heated plate at 37°C. The embryos were then washed and incubated at 37°C in an atmosphere of 5% O_2, 5% CO_2, and 95% N_2 until they were transferred in Global medium.

An inverted microscope at 400× magnification was used to examine embryos 1–2 h after warming, and degrees of damage were calculated. The levels used to classify embryo damage were 0, 1–25, 26–50, and >50%. We selected embryos with <26% damage. Selected embryos were cultured for an additional 12–18 h. Embryos with equal blastomeres and no detectable fragmentation on the day of embryo transfer were referred to as good-quality embryos. For blastocyst-stage embryos, good quality was characterized by the presence of many tightly packed cells in the inner cell mass. We transferred two embryos per patient.

After embryo transfer, patients were monitored. A urine β-hCG test was performed 14 days after embryo transfer, and clinical pregnancy was verified when a gestational sac was detected via pelvic ultrasound. Both of these parameters were used to confirm pregnancy success.

Groups

After eligibility criteria were applied, 315 patients were divided into two groups according to the interval between biopsy and embryo transfer: group 1 (early, n=134—an interval of <90 days) and group 2 (late, n=181—an interval of >90 days and up to 180 days).

Variables

The main variable included the pregnancy success rate after embryo transfer in two moments: early (<90 days) and late (90–180 days). Also, we analyzed the other variables such as age (years), body mass index (BMI), type of assisted reproductive technology (ART), and endometrial preparation through the clinical chart in the medical records of Clinica Gera.

Bias

This study is retrospective based on the medical records. Also, we did not include a group without endometrial scratching. Other bias was the lack of live pregnancy.

Statistical analysis

A power analysis was performed between the early and late groups with 240 patients based on pregnancy success rates and found a difference of 50 and 30% between the groups (α=0.40). For each group with 80% power (1-β), there was a minimum of 120 females per group. Parameters were evaluated using χ^2 tests, and Pearson's coefficients (r) were calculated to determine correlations; Student's t-test was also used for statistical analysis. We analyzed the time between endometrial biopsy and embryo transfer. We considered the assessed outcome (pregnancy success or failure). We also used multilevel multivariate regression analysis to evaluate the confounding effects of various variables, such as age, BMI, type of ART, and endometrial preparation, on the results.

RESULTS

Participants

A flowchart of the study patients is shown in Figure 1. Initially, 450 patients were included. Later, 135 patients who did not meet the inclusion criteria were excluded: patients with endometritis or functional micropolyps (n=40), systematic arterial hypertension (n=33), psychotropic drugs (n=28), hyperprolactinemia (n=12), systemic erythematosus lupus (n=9), thyroid dysfunction (n=8), and diabetes mellitus (n=5). The final number of patients (315 women) was divided into two groups as follows: (1) early group (n=134) and (2) late group (n=181).

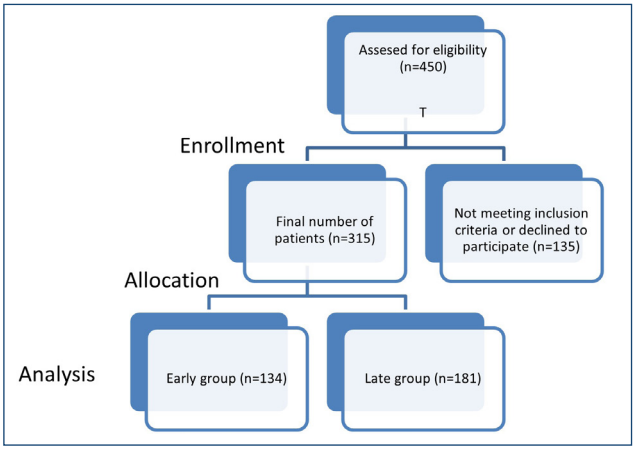

Figure 1. Flowchart of the study.

The clinical characteristics of the patients are summarized in Table 1. The two groups were similar with respect to age, BMI, type of ART, and endometrial preparation.

Main results

Data regarding pregnancy success rates in the two groups are presented in Figure 2. The overall positive pregnancy rate regardless of the interval between biopsy and embryo transfer was 49.5% through positive pregnancy test. When the two groups were analyzed independently, it became evident that pregnancy rate was influenced by the number of days between endometrial scratching and embryo transfer.

The mean number of oocytes retrieved was 6.85±5.41 and 7.02±4.52 in early and late groups, respectively (p=0.57). The mean number of produced embryos available was 4.25±0.78 and 3.92±0.94 in early and late groups, respectively (p=0.35). The number of embryos transferred was fixed in two for each group.

Superior results were obtained for the group with an interval between biopsy and embryo transfer of less than 90 days relative to the group with an interval of more than 90 days (p=0.04). Positive pregnancy test rates of 58.5 and 43.4% were observed in late and early groups, respectively. The odds ratio for positive test and clinical pregnancy success was 1.63 (95% confidence interval: 1.04 to 2.55) and 2.48 (95% confidence interval: 1.46–3.50), respectively. The number of patients with clinical pregnancy for early and late groups was 52 (134) and 48 (181), respectively.

Other analyses

The results of the multivariate regression analysis indicated that age, BMI, type of ART, and endometrial preparation did not significantly influence the study results.

DISCUSSION

Key results

The best time for embryo transfer is a dilemma in the reproductive studies, but the endometrial scratching is considered to enhance the reproductive outcomes of embryo implantation[8]. In fact, our main result was that the pregnancy rate when analyzed for the group with an interval between biopsy and embryo transfer of less than 90 days was significantly higher compared to the group with an interval of more than 90 days. This finding has clinical application and relevance for deciding the best moment for embryo transfer. Also, the influence of clinical characteristics such as age, BMI, type of ART, and endometrial preparation on the results was similar between the groups analyzed.

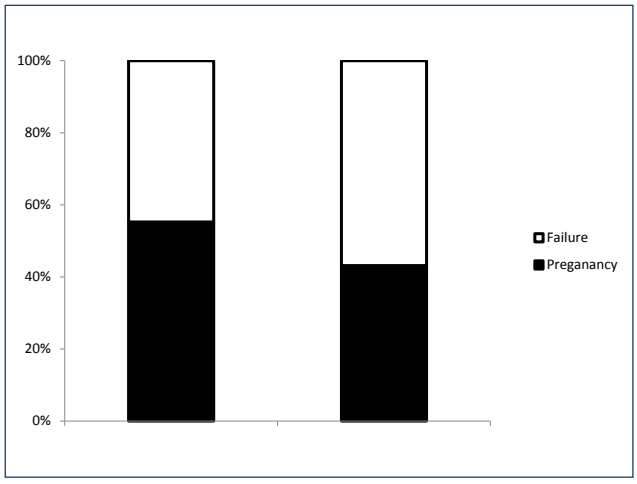

Figure 2. Evaluations of pregnancy success for the two groups: group 1 (n=134) (early—<90 days and group 2 (n=181) (late—>90 days and up to 180 days). p=0.04. The χ^2 test was applied.

Table 1. Clinical features of patients in the two groups.

Endometrial biopsy	<90 days	>90 days and up to 180 days	P
Number of patients	134	181	
Clinical aspects			
Age (years)	36.9±0.1	37.1±0.9	ns
BMI (years)	23.4±0.8	23.2±0.5	ns
Type of ART, n (%)			
ICSI	134 (100)	181 (100)	ns
Type of endometrial preparation, n (%)			
Natural cycle	22 (16.4)	27 (14.9)	ns
Progesterone supplementation	112 (83.6)	154 (85.1)	
Pregnancy rate, %	58.5	43.4	0.04

BMI: body mass index (km/m^2); ART: assisted reproductive technology; ICSI: intracytoplasmic sperm injection; ns: nonsignificant. Student's t-test was applied for age and BMI variables and the other variables were analyzed by χ^2 test.

Interpretation

Our results may enforce this idea by indicating that early embryo transfer (<90 days) is better than late embryo transfer for pregnancy success. Amaral et al.[9] reviewed the influencing factors of pregnancy loss and survival probability of clinical pregnancies through ART and some factors such as maternal age, controlled ovarian hyperstimulation protocol, cycle type, and serum hCG level 14 days after transfer. Bashiri et al.[10], who reviewed on recurrent implantation failure (RIF), suggested a new initial step in approach to patients with RIF, as in this study. A 2022 Cochrane review[11] calls for more trials, suggesting that there is only moderate-quality evidence that endometrial injury done between day 7 of the previous cycle and day 7 of the embryo transfer cycle can lead to increased clinical pregnancy and live birth rates in women with previous embryo transfer[12,13].

Our results of clinical characteristics in endometrial biopsy between the groups less than 90 days and the time between 90 and 180 days did not show differences between the groups with the characteristics of mother's age, BMI, type of endometrial preparation, natural cycle, and progesterone supplementation. Our results are not in agreement with some clinical characteristics in the literature, such as maternal age, that the older the mother, the lower the pregnancy success rate and the higher the BMI, and hence the lower the pregnancy success rate[9,14]. Another study concluded that infertility duration, endometrial thickness, and number of embryos transferred might affect the live birth rate after frozen embryo transfer among young women[15].

Certain investigators have hypothesized that the expression of inflammatory genes after mechanical damage may be responsible for the observed increase in endometrial receptivity, which is a key factor regulating blastocyst implantation[6-9]. In fact, mechanical trauma to the endometrium alters gene expression and the local immune system (via monocyte recruitment), enhances the secretion of growth factors, and makes the endometrium more receptive to implantation[6]. However, other investigators have concerns regarding these effects due to certain divergent results[10]. A possible explanation may be that the aforementioned process is time dependent and transient. In fact, it is not only time of frozen oocyte as important factor, but the endometrial preparation may be other that influences the final results.

Nastri et al.[13] evaluated the effectiveness and safety of endometrial injury prior to embryo transfer in women undergoing treatment with ART. These authors included 591 patients from 5 different trials and concluded that endometrial injury prior to the embryo transfer cycle improves clinical pregnancy and live birth rates in women undergoing ART but that inflicting endometrial injury on the day of oocyte retrieval is not advised since that approach appears to significantly reduce clinical and ongoing pregnancy rates. However, Potdar et al.[8] and Nastri et al.[13] did not describe how type of endometrial injury or the length of time between endometrial injury and embryo transfer may influence blastocyst implantation. We believe that endometrial biopsy is the preferred approach because a biopsy helps identify certain microscopic endometrial causes of infertility, such as chronic endometritis (CE), which is a condition involving the breakdown of the peaceful coexistence between microorganisms and the host immune system in the endometrium[16,17]. Unfortunately, in most cases, CE produces no noticeable signs or only mild symptoms. Therefore, this entity may be neglected by gynecologists and pathologists due to its mild clinical manifestations and the time-consuming microscopic examinations necessary for its diagnosis. Based on diagnostic criteria for CE, the prevalence of this condition is approximately 11.1% in the general population[18], and it is highly prevalent among infertile women[14-18].

Generalizability

Although two independent pathologists examined our biopsy samples for endometrial quality, this procedure was not a primary outcome of our study. Also two independent embryologists analyzed the quality of embryo after the frozen procedures.

Limitations

Our study design was retrospective and observational. In addition, our study is neither prospective nor randomized, and these aspects of our investigation may have influenced our results. Also, the question is about the influence of endometrial quality on the results was not possible with our protocol.

CONCLUSION

The early transfer of embryos (<90 days) may produce better results with a high rate of pregnancy. Further studies are necessary to prove that the length of time between endometrial injury and embryo transfer has a critical influence on pregnancy success and to identify the mechanism involved in this effect.

AUTHORS' CONTRIBUTIONS

JU: Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. **RMS:** Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. **DE:** Data curation, Formal Analysis, Project administration, Writing – original draft, Writing

– review & editing. **FMHC:** Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. **ECAV:** Writing – original draft, Writing – review & editing. **JMSJ:** Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. **ECB:** Writing – original draft, Writing – review & editing.

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