



Oocyte pick up day mucus removal: Does it have a positive effect on pregnancy rates?

Oosit toplama zamanı mukus temizliği: gebelik üzerine pozitif etkili midir?

Cervical mucus removal

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Öz

Amaç: Embriyo transfer zamanı yapılan servikal mukus temizliği transfer sırasında uterin kontraksiyonlara neden olacağı için oosit toplama zamanında (OPU) yapılması daha iyi gebelik sonuçları ile ilişkilidir. Biz çalışmamızda OPU günü servikal mukus temizliği, embriyo transfer günü servikal mukus temizliği ve hiç servikal mukus temizliği yapılmamasının üç gruptaki gebelik üzerine etkisini araştırdık. Gereç ve Yöntem: Çalışmayı prospektif match control olarak düzenledik. 131 siklus mukus temizliği OPU günü (64 siklus) ve Embriyo Transfer günü (67 siklus) yapılan hasta ile 411 siklus servikal mukus temizliği yapılmayan hastaları birbiri ile karşılaştırdık. Bulgular: Sırasıyla OPU günü mukus temizliği, embriyo günü mukus temizliği ve mukus temizliği yapılmayan grupların gebelik oranları %40,6, %29,9 ve %23,1 olarak hesaplandı ve gruplar arasındaki fark istatistiksel olarak anlamlı saptandı (p=0,009). Tartışma: OPU günü yada embriyo transfer günü mukus temizliği gebelik oranlarını artırmaktadır. Mukus temizliği potansiyel olarak servikal travmayı ve uterin kontraksiyonu azalttığından rutin klinik kullanım pratiğine dahil edilebilir ve böylece IVF/ICSI başarısı artırılabilir.

Anahtar Kelimeler

Servikal Mukus; Embriyo Transfer; Gebelik Oranları; Transfer Zamanı; Oosit Toplama

Abstract

Aim: Mucus removal that would be done on the day of embryo transfer could be done on Oocyte pick up (OPU) day, and this practice might be related with better pregnancy rates since it is unlikely to cause uterine contractions during the embryo transfer. We compared the effects of mucus removal done on OPU day, transfer day or no removal at all on success rates of the treatment in three groups. Material and Method: We designed a prospective match control study. There were 131 cycles in which mucus removal was done on either OPU day or embryo transfer day (64 and 67 cycles respectively) and in 411 cycles mucus removal was not done, and these patients constituted the control group. Results: After mucus removal on OPU day, mucus removal on embryo transfer day and no mucus removal done at all, pregnancy rates were calculated as 40,6%, 29,9%, and 23,1% respectively and the difference among the groups was statistically significant (p=0,009). Discussion: Mucus removal on OPU day or on embryo transfer day improves pregnancy rates. It can be included in the routine practice, since it may potentially minimize cervical trauma and uterine contractions and thus improve success in IVF/ICSI treatments.

Keywords

Cervical Mucus; Embryo Transfer; Pregnancy Rates; Transfer Timing; Oocyte Pick Up

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Introduction

Currently, Assisted Reproductive Technologies help countless couples become parents. These techniques achieve success in 25 to 40% of all procedures [1-3]. As the eventual goal is the realization of a healthy baby, researchers continuously search for reasons of failure and the ways to overcome these.

The success of embryo transfer which is the final step in IVF/ICSI treatments depends on additional factors other than endometrial receptivity and the quality of embryos. Ultrasound guidance, myometrial contractions, type of transfer catheter or the mode of loading, dummy transfer, the site that the embryos were placed, presence of cervical mucus and /or blood, a blood-stained catheter after transfer or an embryo stuck in the catheter are some such factors [4-6].

Sperms need to go through vagina and cervix and reach upper genital system for fertilization and to achieve this they have to penetrate into the cervical mucus (CM) which is a hydrolyzed gel containing various forms of mucin. CM is maximally hydrated during ovulation. Its pores are smaller than the heads of the sperms and sperms have to pass through these pores to reach the upper genitals. Higher rates of in vivo or in vitro pregnancies are related to the sperms which managed to penetrate through this barrier [7,8].

Occasionally there is mucus smeared on the cervix impeding the embryo transfer. Transferring the embryos may be complicated when the cervix is covered with mucus. It is considered that as the inner catheter used for embryo transfer is made of soft material, the cervical catheter might obliterate the catheter, impede the transfer of embryo(s) to the desired location or cause damage to the embryo(s) [2,3,9].

Conversely, removing the mucus which acts as a natural lubricant might complicate transfer [10]. Additionally, poking the cervix or the endocervix during the mucus removal might trigger uterine contractions and lead to decreased pregnancy rates [4]. We anticipated that the mucus removal that would be done on the day of embryo transfer could be done on OPU day and this practice might be related with better pregnancy rates since it is unlikely to cause uterine contractions during the embryo transfer. In this study, we compared the effects of mucus removal done on OPU day, done on transfer day or no removal at all on success rates of the treatment in three groups. We also performed a thorough review of the literature and discussed the influences on routine practices.

Material and Method

Study Design:

In order to evaluate the effect of timing of mucus removal on pregnancy rates, we designed a prospective match control study in which the records of 447 IVF/ICSI patients who had a total of 542 procedures were compared. There were 131 cycles in which mucus removal was done on either OPU day or embryo transfer day (64 and 67 cycles respectively) and in 411 cycles mucus removal was not done, and these patients constituted the control group. The study was conducted in a university setting, and the primary outcome was set as pregnancy rate.

Patients:

This study was a prospective match control study conducted on the records of patients who were treated in Istanbul Univer-

sity, Cerrahpasa School of Medicine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility. Mucus removal was done either before the OPU or the embryo transfer, and these patients were compared with those who had OPU and embryo transfer without any mucus removal at all. Institutional Review Board approved the study. There were 447 patients in total, and these patients underwent 542 IVF/ICSI cycles. In mucus removal group which consisted of patients who had mucus removal either during OPU or embryo transfer, in 64 cycles, mucus removal was done before OPU, and in 67 cycles it was done before embryo transfer. In 411 cycles no mucus removal was done at all. Cases in which embryo transfer was canceled were excluded from the study.

Stimulation protocols:

In agonist protocols, leuprolide acetate (Lucrin, Abbott) was used as Gonadotropin-Releasing Hormone (GnRH) agonist and in antagonist protocol GnRH antagonist cetrorelix (Cetrotide, Serono) was used. Urinary or recombinant gonadotropin doses were arranged according to follicle dimensions measured by ultrasonography and serum E2 levels. Ovulation trigger of the oocytes was done by an injection of 10000IU urinary hCG (Pregynl, Organon) or 250mcg recombinant hCG (Ovitrelle, Serono). Oocyte retrieval was performed 36 hours after the hCG injection under general anesthesia and ultrasound guidance.

Embryo selection and transfer:

All of the embryos were obtained by applying ICSI method on the oocytes. Patients under 35 received a single embryo while patients over 35 and any patient with at least 2 prior failed IVF/ICSI trials had 2 embryos transferred. All embryo transfer procedures were performed exclusively by certified staff physicians in the unit. All oocyte retrievals and embryo transfers were done in the same operating room. (Patient admission and follicle measurements were done in a separate room). Patients were put in a lithotomy position on a gynecologic examination chair. Cervix was made visible by using a metal bivalve speculum, and external cervical os was cleaned with a saline solution, and cervical mucus was removed by using a cotton swab and a polyvinyl propylene catheter mounted on a 10 cc syringe to create negative pressure and aspirate the mucus. All of the embryo transfers were done under ultrasound guidance. After the transfer is complete, each catheter tip was checked for blood stain or residual mucus. They were inspected with a microscope to rule out any retained embryos in the catheter, as well. Vaginal progesterone (Crinone gel % 8, Serono) was given to all patients after each transfer for luteal phase support. Serum bHCG level was measured for detecting the pregnancy 12 days after the transfer.

Statistical Analysis:

Statistical analysis was performed using the SPSS (Statistical Program for Social Sciences, Chicago, IL, USA) program. Data were characterized by means, standard deviations, and percentages. A p-value <0.05 was considered as statistically significant.

Limitations of the study:

The study design was a prospective match control study, thus any randomization was irrelevant. Patients in the groups were treated with long agonist protocol and antagonist protocol. Lack of other types of treatment protocols is a limitation of the study. In our IVF unit, patient follow-up is done by a team of specialists instead of a single physician. Thus, follicle measurements, mucus removal procedures, and embryo transfers were not performed by the same person. The patients were enrolled in the study and divided into groups should the specialist taking part in their treatment procedures had similar success rates of embryo transfer.

Results

A total of 447 patients and 542 IVF/ICSI cycles undergone by these patients were included in the study. OPU day cervical mucus removal was done in 64 patients, mucus was removed on the embryo transfer day in 67 cycles, and no removal at all was attempted in 411 cycles. Demographics and biochemical test results of the patients in the groups are given in Table 1. Three groups were similar in mean age, BMI, gravidity, parity and the duration of infertility (p values: 0.20; 0.42; 0.18 and 0.23 respectively). Mean values of total duration of ovarian hyperstimulation in each group were $9,07 \pm 1,9$; $8,9 \pm 1,9$; $9,5 \pm 1,9$

Table 1. Demographics, laboratory parameters, and data on cycles of all three groups

	Mucus removal on OPU day (n:64) mean±sd	Mucus removal on embryo transfer day (n:67) mean±sd	No mucus removal (n:411) mean±sd	p
Age	32,92±4,6	32,25±5,25	32,52±5,05	0,20
BMI ¹	26,8±5,4	25,9±4,7	25,7±4,6	0,42
Gravidity	0,0±0,0	0,9±0,5	0,8±0,4	0,18
Infertilityduration	6,5±3,7	8±4,6	7,6±4,5	0,23
FSH ²	5,9±2,7	6,6±2,6	6,9±3,1	0,38
LH ³	4,2±2,7	5,0±2,7	5,4±3,4	0,89
Estradiol	47,1±6,1	52,3±5,1	47,1±1,4	0,80
Prolactin	16,8±8,6	18,1±8,6	18,7±13,4	0,06
TSH ⁴	1,97±1,4	2,8±0,5	2,3±2,5	0,18
AMH ⁵	3,5±2,3	3,4±3,9	3,6±4,0	0,18
Duration of induction	9,07±1,9	8,9±1,9	9,5±1,9	0,41
Total drugdose	2518±1048	2215±866	2527±975	0,25
Oocytesretrieved	8,1±4,1	7,0±5,1	7,6±4,7	0,39
Embryostransferred	1,5±0,8	1,4±0,6	1,5±0,5	0,22

1BMI:Body Mass Index; 2FSH: Follicle Stimulating Hormone; 3LH:Luteinizing Hormone; 4TSH:Thyroid Stimulating Hormone; 5AMH:Anti Mullerian Hormone

Table 2. Pregnancy counts and rates of all three groups

	Mucus removal on OPU day (n/%)	Mucus removal on embryo transfer day(n/%)	No mucus removal (n/%)	p	p ¹	p ²	p ³
Pregnancy	26 (40,6%)	20 (29,9%)	95 (23,1%)	0,009	0,197	0,003	0,232

p1 : Comparison of OPU day cervical mucus removal group with embryo transfer day mucus removal group

p2 : Comparison of OPU day cervical mucus removal group with no mucus removal group

p3 : Comparison of embryo transfer day cervical mucus removal group with no mucus removal group

*Pearson Chi-square test was done.

days respectively and the difference was insignificant among the groups (p=0,41). In our study, after mucus removal on OPU day(Table 2), mucus removal on embryo transfer day and no mucus removal done at all, pregnancy rates were calculated as 40,6%, 29,9%, and 23,1% respectively and the difference among the groups was statistically significant(p=0,009).

Discussion

It is anticipated that removing cervical mucus before the embryo transfer will facilitate the translocation of the embryo into the uterine cavity and thus improve the rates of clinical pregnancy rates and therefore live birth rates.

In approximately 30% of all patients undergoing embryo transfer experience powerful uterine contractions which lead to unfavorable results of IVF treatments. Inducing uterine contractions (>5 per minute) during embryo transfer may result in impaired embryo implantation[11]. In addition, the presence of blood and/or mucus, bacterial contamination, avoiding trauma and uterine contractions are all factors related to optimal rates of implantation and pregnancy[12].

Removal of cervical mucus during embryo transfer improves rates of implantation and pregnancy independently from embryo implantation. However, cervical mucus removal may trigger undesired uterine contractions, as well[10].

A literature review revealed that in a study, frequency and directions of uterine contractions were compared in fresh transfers after IVF treatment. In the prospective cohort study, 286 women were investigated. In embryo transfers after IVF, frequency and directions of uterine contractions were evaluated 5 minutes before, 5 minutes and 60 minutes after the embryo transfer procedure. In the group with increased frequency of contractions at 5 minutes after the procedure, pregnancy rates were lower, and the difference was statistically significant (p=0,006). The authors concluded that frequency of uterine contractions 5 minutes after the embryo transfer was significant in determining the results of IVF and could be useful in selecting the patients who would benefit from muscle relaxants to improve the IVF results[13].

A meta-analysis focussed on the effectiveness of oxytocin antagonist infusion on embryo transfer day. Out of 123 studies taken into consideration, 3 randomized controlled trials were eligible for the meta-analysis. Atosiban (Tractocile, Ferring Arzneimittel, Kiel, Germany) is a well-known antagonist of oxytocin/vasopressin V1a receptor, and it is usually used for preventing preterm delivery. In the study, researchers investigated the effect of atosiban on IVF cycles, as uterine contractions are associated with unfavorable IVF results. Meta-analysis revealed that

administration of oxytocin antagonist on embryo transfer day increased implantation rates but clinical pregnancy rates and abortion rates remained the same. Furthermore, they suggested that prospective randomized trials with higher sample sizes should be done to confirm their conclusions[11]. Authors investigated the effect of blood and mucus on the catheter tip after the embryo transfers on the rates of implantation and pregnancy. A total of 8311 embryo transfers were assessed retrospectively. Presence of blood stain on the tip of the

catheter was associated with a decrease in the rates of implantation, clinical pregnancy, and live birth. On the other hand, a mucus remnant on the tip was considered a simple contamination which has practically no effect on pregnancy rates. Freely floating blood clot fragments in the endometrial cavity which result from cervical trauma were anticipated to trigger uterine contractions and prevent the embryo from implanting on the desired site and such a phenomenon would lead to significantly decreased rates of implantation and clinical pregnancy[6].

In a study comprising 317 patients and 428 cycles done, mucus removal done before the embryo transfer was compared with a control group which did not receive any intervention. Mucus removal immediately preceding the embryo transfer did not increase the live birth rates significantly. However, the authors suggested the practice of mucus removal even if the overall contribution to success was trivial since it is a simple and inexpensive procedure[11]. Similarly, we found that performing mucus removal and doing this procedure on a day other than the embryo transfer day could increase pregnancy rates.

Furthermore, in a systematic review and meta-analysis covering 8 randomized controlled studies containing 1715 IVF/ICSI patients, the effect of cervical mucus removal before the embryo transfer on implantation and pregnancy rates was investigated. Studies included in the review were low to intermediate quality, and the researchers concluded that mucus removal done before the embryo transfer in IVF/ICSI patients had a very small benefit. The results also suggested that implantation, clinical pregnancy, and live birth rates were similar[4].

In another study, the authors stated that mucus remnants on the tip of embryo transfer catheter was associated with lower implantation and pregnancy rates[1,10,12]. Although contradicting views and publications coexist in the literature, we maintain the view that cervical mucus removal may improve the clinical pregnancy rates[1,10].

In our study, OPU day mucus removal group was compared with no removal group, and pregnancy rate improved significantly in the first group ($p=0,003$). Similarly, the embryo transfer day mucus removal group had better pregnancy rates compared with no removal group, but this difference did not reach statistical significance ($p=0,232$).

As a conclusion, mucus removal on OPU day or on embryo transfer day improves pregnancy rates. Although the difference in the pregnancy rates between OPU day and embryo transfer day mucus removal is not statistically significant, it can be included in the routine practice, since it may potentially minimize cervical trauma and uterine contractions and thus improve success in IVF/ICSI treatments.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and

its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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