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ASSISTED REPRODUCTION TECHNOLOGIES

Comparison of pregnancy outcomes of cervical mucus washing with physiologic saline solution or G-Rinse medium solution, in elective single-embryo transfers

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Abstract

Aim To compare the pregnancy outcomes between physiologic saline and G-Rinse medium solution for cervical mucus washing, in fresh elective single-embryo transfers (ET) in women under the age of 37.

Material and methods This was a retrospective data analysis performed in a single in vitro fertilization (IVF) center between February 2018 and November 2021. Women younger than 37 years who underwent single elective ET were included and all women had anti-Mullerian hormone (AMH) levels ≥ 1.5 ng/ml. Age, body mass index (BMI), AMH levels, and pregnancy outcomes as clinical pregnancy rate (CPR) and live birth rate (LBR) were analyzed.

Results Study population consisted of 75 women in the G-Rinse medium solution group and 97 women in the physiologic saline group. Clinical pregnancy rate was 58.7% and 61.9% in the G-Rinse medium solution group and saline group, respectively ($p = 0.673$), and LBR was calculated as 41.3% and 47.4% in the G-Rinse medium solution group and saline group, respectively ($p = 0.430$). A log-binomial regression model was used and the model was adjusted for BMI to evaluate the effect of the cervical mucus washing method on the pregnancy outcomes. There was an estimated 5% decrease in the relative risk for CPR in the G-Rinse medium solution group compared to the saline group (95% CI: 0.74 to 1.2, $p = 0.673$). There was an estimated 13% reduction in the relative risk for LBR in the G-Rinse medium solution group compared to the saline group (95% CI: 0.62 to 1.23, $p = 0.430$). They were both statistically not significant.

Conclusion In our study, the replacement of using G-Rinse medium solution to physiologic saline solution for cervical cleaning did not change CPR and LBR outcomes. Using physiologic saline solution can be a good alternative approach for ectocervical washing during embryo transfer in selected population because of its lower costs, easy accessibility, and common use.

Keywords Embryo transfer · Mucus · Cervix · Ectocervical washing · Physiologic saline solution · G-Rinse medium solution · Clinical pregnancy rate · Live birth rate · In vitro fertilization

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Introduction

Embryo transfer (ET) is among the final crucial steps determining the success of assisted reproductive technologies (ART). This procedure itself involves many steps that must be carried out with caution and care. There are articles in the literature about how to perform successful ET [1, 2]. The American Society for Reproductive Medicine (ASRM) published a guideline about optimal transfer techniques on evidence-based literature. They concluded that removal of mucus from the cervical canal prior to ET improved clinical pregnancy rates (CPR) and live birth rates (LBR) with grade B evidence, which seems good enough to support removal of cervical mucus [3] .

Cervical mucus is a product of the cervical gland in response to estrogen; the characteristics of mucus can change as sticky, watery, or creamy due to hormone levels. Mucus consists of insoluble mucin and soluble watery components as lipids, fatty acids, prostaglandins, trace elements, proteins, enzyme inhibitors, and immunoglobulins [4–6] . Mucus is a hydrogel including 90–95% water and heterogenous mixture of abovementioned ingredients as the remaining part.

Physiologically, cervical mucus plays a protective reservoir role for sperms during natural fertilization [7] . However, cervical mucus might negatively impact the success rates of intrauterine insemination (IUI) or in vitro fertilization (IVF) treatments. Mucus can decrease the success rates of the treatment in various ways as follows:

- Cervical mucus can block the transfer catheter tip and can create a mechanic barrier to the hole in the opening tip of the inner catheter; therefore, embryos can stick to the wall of the transfer catheter and might be retained.
- Cervical mucus can contaminate the intrauterine environment because mucus stuck to the transfer catheter may carry microorganisms into the uterine cavity and intervene with implantation.
- In addition, the chemokines from the cervical mucus, when carried to the endometrium, can initiate unwanted uterine contractions.
- Finally, cervical mucus might also result in displacement of the embryo during insertion, expulsion, or withdrawal of catheter [1, 8, 9] .

Therefore, it is recommended to remove the cervical mucus before inserting the transfer catheter during the ET procedure.

According to the committee opinion by ASRM, flushing or cleansing the cervix and vagina with cotton swab or gauze sponge using medium or saline after speculum placement at the beginning of ET is recommended as a standard embryo transfer protocol. A survey of the Society for Assisted Reproductive Technology (SART) filled by medical directors revealed that 96% of directors cleansed cervix whereas 4% did not. The cleansing method was questioned among clinicians: 17% were cleansing with saline, 78% were using mediums, 2% were using other solutions that were not indicated, and 3% gave no response [3].

The medium generally chosen to wash the ectocervix is the user-friendly G-Rinse medium solution (Vitrolife, Sweden) which is a bicarbonate-buffered salt solution and contains gentamicin, and has the same osmolality as culture mediums. The reason why we are using medium solution (G-Rinse medium solution) for ectocervical washing during embryo transfer is that it provides a stable, clean environment while the ET catheter is passing through the cervical canal and it is tested as safe for IVF.

The ectocervical washing to remove cervical mucus prior to ET was routinely performed with pre-warmed G-Rinse medium solution in our institution. When the COVID-19 pandemic outbreak was declared in March 2020 by the World Health Organization (WHO) [10], the island where our institution is located went into a national lockdown and restrictions were made for most of the imported and exported supplies. Because of the difficulties in accessing certain medical supplies and due to logistic reasons after the COVID-19 national lockdown, G-Rinse medium solutions were depleted and we decided to shift to using physiologic saline solution to wash the ectocervix in order to remove cervical mucus during the ET procedure, instead of the G-Rinse medium solution. Thus, cervical mucus has been removed with ectocervical washing with either physiologic saline (0.9% NaCl) solution or with G-Rinse medium solution at our institute depending on time as before or after pandemic.

To the best of our knowledge, no paper has been published in the literature investigating whether removing cervical mucus with washing the ectocervix with physiologic saline or G-Rinse medium solution is better in terms of pregnancy outcomes. Because of this uncertainty, we aimed to compare the pregnancy outcomes regarding CPR and LBR between physiologic saline and G-Rinse medium solution for cervical mucus washing in fresh elective single-blastocyst transfers performed to women < 37 years of age.

Material and methods

This study was conducted in an international IVF Center in Northern Cyprus between February 2018 and November 2021 with retrospective cohort data analysis of 172 single elective blastocyst transfer cycles performed to women < 37 years of age.

Local institutional review board approval was obtained from the Ethical Committee of Yakin Dogu University (YDU/2022/ 104–1549). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The eligible patients were asked for oral and written consents in order to obtain their permission for their data to be included in the analysis and allowing the use of their blinded clinical data for research purposes.

All treatment procedures including ovarian stimulation, oocyte retrieval, and ETs were done by the same attending physicians (AE and BA) with the same standardized technique for years, with similar live birth rates. Authors TG, HGC, JY, and EB did not participate in the recruitment or clinical care of patients. They were responsible for data analysis, interpretation, and writing of this manuscript.

The patients having the indication for IVF/intracytoplasmic sperm injection (ICSI) due to infertility with unexplained and anovulation reasons were selected. We only included the patients who had cervical mucus during the transfer procedure in the study and excluded the patients who did not have any cervical mucus. IVF with preimplantation genetic testing for aneuploidy (PGT-A) cycles, frozen cycles, donor cycles, and ≥ 2 embryo transfers were excluded from the study. The patients with ≥ 37 years of age, AMH < 1.5 ng/ml, and etiologies of infertility other than unexplained causes or anovulation were excluded. Recurrent implantation failure and uterine or tubal abnormalities were also excluded to obtain more homogenous groups. In addition, the partners of the participants had normal sperm analysis whereas those with abnormal sperm parameters or necessity of any testicular interventions were excluded.

Age, body mass index (BMI), anti-Mullerian hormone (AMH) levels, metaphase II (MII) oocyte number, 2 pronuclear (PN) embryo number, blastocyst (day 5) number, endometrial thickness measured by transvaginal ultrasonography (USG) on the day of progesterone initiation, embryo-fundus distance measured during ET, quality of embryo to be transferred, embryo number per transfer, and pregnancy outcomes as CPR and LBR were analyzed from the hospital records in the study.

Controlled ovarian stimulation (COH)

Ovarian stimulation cycles were routine standardized protocols with gonadotropins and all patients were prepared with gonadotropin-releasing hormone (GnRH) antagonist protocol. Ovarian stimulation was monitored with transvaginal ultrasound (USG). Ovulation was triggered with choriogonadotropin alpha 250 µg/0.5 ml (Ovitrelle®, Merck, Germany) when more than 3 follicles were ≥ 18 mm. Oocyte retrieval was performed transvaginally 35–36 h after trigger. All sperms were selected by the swim-up technique prior to ICSI. All MII oocytes were fertilized through ICSI by the embryologist. All of the embryos were followed up with a time-lapse system (EmbryoScope®, Vitrolife, Sweden). Fertilization was assessed 18–20 h after ICSI and morphological scores were assessed on day 3 and day 5 of the embryo development [11] .

Embryo transfer technique

Embryo transfers were performed in a standardized fashion at our center. During the ET procedure, after application of speculum, a filling tube (Mixing Kwill, Henley's Medical, UK) attached to a 10-ml syringe was used for ectocervical washing to remove cervical mucus. It was routinely performed with pre-warmed G-Rinse medium solution in our institution. After the outbreak of the COVID-19 pandemic, as it was not possible to supply G-Rinse medium solution due to the restrictions, it had to be replaced with physiologic saline solution. Thus, cervical mucus had been removed by ectocervical washing with either physiologic saline (0.9% NaCl) solution or with G-Rinse medium (Vitrolife, Sweden) solution depending on time as before or after pandemic. Ten milliliters of either pre-warmed G-Rinse medium or physiologic saline solution was given via the filling tube to wash ectocervix. While washing solution was being given inside, the tube tip did not contact external cervix in order to prevent any unwanted uterine contractions. Care was taken to aspirate the solution back from the lower blade of the speculum, and the cervix was not touched. After washing, any persistent mucus was aspirated with a sterile Pipelle cannula (CooperSurgical®, Inc) without touching the cervix. Any mucus on the external cervical ostium was removed this way before inserting the transfer catheter.

Embryo was loaded to a Wallace catheter (Soft 23 cm, Classic, 1816N, CooperSurgical®, Inc) with air-embryo-air-medium order. The total volume to be transferred was restricted to 0.2 ml. All ETs were performed under transabdominal USG guidance. The embryos were expulsed in the upper middle uterus approximately 10–20 mm from the fundus via the soft catheter, avoiding to touch the fundus. The distance between the uterine fundus and air bubble (which was assumed as an indirect noninvasive marker of embryo) seen on transabdominal USG imaging immediately at the time of ET was accepted as embryo fundal distance in millimeters. The catheter was examined for any retained embryos immediately.

Evaluation of pregnancy

Beta-human chorionic gonadotropin (hCG) levels were measured 12 days after the ICSI procedure. Beta-hCG level

lower than 50 IU/ml was accepted as negative pregnancy. Beta-hCG measurement was repeated 48 h later. If level was decreasing, it was termed as "biochemical pregnancy." Five weeks after ET, the presence of any intrauterine gestational sac with fetal cardiac activity was accepted as "clinical pregnancy." Any pregnancy located outside the uterine cavity was named "ectopic pregnancy." Intrauterine pregnancy was named "miscarriage" when terminated before 20 weeks of gestation. Any pregnancy terminated beyond 20 weeks was termed as "birth." The birth of a live fetus was named "live birth." Clinical pregnancy per ET was named CPR while live birth per ET was named LBR.

Statistical analysis

Statistical analysis was performed using the SPSS version 22 (Statistical Package for the Social Sciences, IBM, Chicago, IL). Demographic continuous data were characterized by means and standard deviations (SD), and nominal variables were characterized by percentages. Differences in mean values and characteristics between groups were analyzed with the independent samples *t*-test and the chi-square test. The primary outcome measures were CPR and LBR. The variable was washing method whether physiologic saline solution or G-Rinse medium solution. Covariate was BMI, since all patients' age was set to be < 37 years, AMH ≥ 1.5 ng/ml, and endometrial lining to be ≥ 7 mm. In order to assess the relationship between the groups and the pregnancy outcomes as CPR or LBR, a log-binomial regression model is used and the obtained results are presented as beta-coefficients with 95% confidence intervals. A *p*-value ≤ 0.05 was considered statistically significant. According to our knowledge, this study is the first in the literature to compare two washing mediums. The design of this study was retrospective, so the power analysis was performed as post hoc power analysis. The power of the sample size was calculated using G*Power 3.1 software. A post hoc power analysis was performed, using the same method described in Eskandar et al.'s study, to compute achieved power of the current sample ($n = 172$) for significant variables, and the minimum statistical power ($1 - \beta$ error probability) was calculated as 0.98 for the comparison of proportions with 0.05 α-error probability [8] . It could be considered that the post hoc power could be low due to similar outcomes between groups. However, our post hoc power analysis results were in favor of high statistical power. Still, further prospective and well-designed studies are needed to obtain more evidence.

Results

There were 172 women to whom single elective ET performed with < 37 years of age and who met inclusion criteria. There were 75 patients (43.6%) to whom pre-warmed G-Rinse medium solution was used and there were 97 patients (56.4%) to whom physiologic saline solution was used.

All women had AMH levels ≥ 1.5 ng/ml, and all endometrial thickness measurements were ≥ 7 mm on ET day. All transferred embryos were of good-quality day 5 blastocysts, and this study included only fresh single-blastocyst transfer obtained from patients' own eggs and partners' own sperms.

Table 1 The comparison of the demographic and clinical characteristics of the patients in whom G-Rinse medium solution and physiologic saline solution used

	G-Rinse medium solution group ($n = 75$)	Physiologic saline solution group ($n = 97$)	*p* value[a]
Age	32.0 ± 3.1	31.4 ± 2.8	0.139
BMI (kg/m^2)	24.0 ± 3.3	23.2 ± 3.9	0.441
AMH (ng/ml)	3.1 ± 1.5	3.5 ± 1.3	0.358
Total gonadotropin dose (IU/l)	2620.9 ± 1013.8	3012.5 ± 840.1	0.102
M2	10.6 ± 4.7	11.2 ± 4.9	0.095
2PN	7.6 ± 3.2	8.3 ± 4.6	0.263
Day 5 embryoblast number	5.7 ± 2.7	6.4 ± 3.5	0.152
Endometrial thickness (mm)	9.7 ± 1.9	9.0 ± 1.9	0.271
Embryo-fundal distance (mm)	13.4 ± 3.3	12.8 ± 4.0	0.371
CPR	44 (58.67%)	60 (61.86%)	0.673
LBR	31 (41.33%)	46 (47.42%)	0.430

BMI, body mass index; *AMH*, anti-Mullerian hormone; *MII*, metaphase II oocytes; *2PN*, 2 pronuclear embryo; *CPR*, clinical pregnancy rate; *LBR*, live birth rate

[a]Independent samples *t*-test and chi-square test were applied

A *p*-value of < 0.05 was considered statistically significant

Patients' demographic and cycle characteristics are given in Table 1. The groups were comparable in terms of age, BMI, AMH, total gonadotropin doses, number of M2, 2PN, and day 5 blastocysts, endometrial thickness, and embryo-fundal distance during ET (Table 1).

As shown in Table 1, pregnancy outcomes were compared between the groups. Clinical pregnancy and live birth rates were slightly greater in the saline group than the G-Rinse medium solution group (61.9% vs 58.7% and 47.4% vs 41.3%, respectively). There was an estimated 5% decrease in the relative risk of clinical pregnancy in the G-Rinse medium solution group compared to the saline group (95% CI: 0.74 to 1.21). This difference was not statistically significant ($p=0.673$). There was an estimated 13% reduction in the relative risk of live birth in the G-Rinse medium solution group compared to the saline group (95% CI: 0.62 to 1.23). This difference was also not statistically significant ($p=0.430$).

After adjusting for BMI, there was an estimated 5% decrease in the relative risk of clinical pregnancy in the G-Rinse medium solution group compared to the saline group (95% CI: 0.74 to 1.21). This difference was not statistically significant ($p=0.673$). Also, after adjusting for BMI, there was an estimated 13% reduction in the relative risk of live birth in the G-Rinse medium solution group compared to the saline group (95% CI: 0.62 to 1.22) without significance ($p=0.418$). The adjusted and unadjusted risk ratios were similar for both outcomes.

Discussion

We sought to evaluate the effect of different solutions used for removal of cervical mucus during ET on pregnancy outcomes in this study. We found that there is no evidence that the risk of clinical pregnancy or live birth in the saline group are lower than the G-Rinse medium solution group. It has been detected that use of G-Rinse medium or physiologic saline solution for this aim did not change the success of IVF treatment regarding CPR and LBR.

It is widely suggested to routinely remove cervical mucus during the ET procedure in order to improve CPR and LBR [8, 12, 13] . However, this clinical question has not been clearly answered in the literature in terms of comparing different solutions to clean the ectocervical mucus before embryo transfer. In a recent study by Sallam et al., removal of cervical mucus was suggested to increase clinical pregnancy outcomes. However, they did not compare the outcomes among different solutions [14].

There is no study in the literature comparing G-Rinse medium solution with physiologic saline solution for the pregnancy outcomes. When the pandemic conditions and restrictions led us to use saline, as we knew that saline was used in previous studies in the literature, we decided to compare pre-warmed G-Rinse medium solution with physiologic saline solution in terms of pregnancy outcomes. Therefore, we investigated whether use of physiologic saline solution instead of G-Rinse medium solution changed CPR and LBR or not. We found that the solution used to wash the ectocervix during embryo transfer — whether physiologic saline solution or G-Rinse medium solution — did not make any significant difference among the selected young age population on pregnancy outcomes.

Even though physiologically cervical mucus plays a protective reservoir role for sperms in natural fertilization, it might negatively impact the success rates of IUI or IVF/ICSI treatments. There are papers indicating that cleansing the cervical mucus during embryo transfer improves the pregnancy outcomes of ART treatments [2, 8, 13]. Simsek et al. found that cervical mucus aspiration prior to IUI cycles improved CPR [15]. In addition, cleansing the cervical mucus prior to transfer catheter insertion was shown to be increasing the likelihood of pregnancy in IVF patients in many studies [8, 13]. On the other hand, in a very recent well-designed meta-analysis by Tyler et al., using cotton swabs, cervical brush, aspirators, or flushing with culture media solution or their combinations was compared and it was shown that there was no significant effect of cervical mucus removal on clinical outcomes or live birth rates [16]. Similarly, a Cochrane review assessing different techniques for preparation prior to ET found no difference in terms of pregnancy outcomes among patients who had their cervical mucus removal or not [17]. However, in this review, only one single study that analyzed cervical mucus removal was included.

There are different methods defined to clean cervical mucus during ET such as removal by a cervical brush, sterile cotton swabs, sterile gauze soaked in saline or transfer medium, vigorous irrigation of the cervical canal, and aspiration of the cervical mucus without flushing [8, 13, 18, 19]. Eskandar et al. found significantly higher pregnancy rates with mucus removal. In their study, they did not flush the ectocervix with any solution, but removed mucus with an aspiration catheter [8]. Mains et al. published a review on optimizing the technique of ET which suggested to remove cervical mucus to overcome plugging and bacterial contamination using a sterile cotton ball, gauze soaked in saline or medium, and a sterile syringe attached to a catheter [12]. Contrary to these studies, in our clinic, we do not use a cervical brush, swab, gauze, or cotton ball in order not to contact the cervix and induce any unwanted uterine contractions or cause any cervical bleeding.

As it is established that cleaning the cervical mucus during embryo transfer increases the pregnancy rates, a well-designed large prospective trial comparing (i) sterile cotton ball, (ii) gauze soaked in saline or medium, and (iii)

ectocervical washing with saline or medium would be more informative to decide on which cervical mucus cleaning method to perform during transfer procedure.

Tiras et al. cleansed the external cervical ostium with saline solution in their study. They stated that finding mucus on the transfer catheter did not affect the pregnancy rates, but women with blood on the transfer catheter had significantly decreased pregnancy rates [20]. We did not detect any blood or mucus on or inside the catheter after the transfer procedure.

Our findings are restricted to women who are less than 37 years of age, with AMH values ≥ 1.5 ng/ml. However, women over the age of 37 or women with lower AMH values are expected to have relatively lower mucus production as their estrogen levels are anticipated to be lower compared to younger women during ovarian stimulation period. Thus, cervical mucus will be less likely to be a problem in this population. Therefore, we speculate that our findings can be applicable to women over the age of 37 or women with lower AMH values. But, in order to study this hypothesis, a well-designed large prospective trial including women below and over the age of 37 is required.

The relatively cheaper solution to wash the ectocervix to remove cervical mucus with physiologic saline solution can be a plausible alternative to G-Rinse medium solution, since it did not result in a statistically significant difference in CPR or LBR. Therefore, it can be considered a safe cleaning method without any compromising effect on pregnancy outcomes. Also, using physiologic saline solution would be more feasible in case of a potential problem in supply chain or logistics, as it is a more easily accessible and commonly used solution compared to other mediums in IVF clinics. However, in order to widely recommend this approach and use in daily practice, prospective trials with higher numbers of patients and trials performing cost-efficiency analyses are required.

Our study has several strengths. This study is the first in the literature that compares two different cleaning solutions to remove cervical mucus during embryo transfer. In addition, our study only included single-embryo transfers. Therefore, further studies comparing these two solutions for washing the ectocervix during embryo transfer to remove cervical mucus should also take into consideration the quantity of the transferred embryos. We did not experience any retained embryos, but such future studies should investigate the presence of any retained embryos. In our study, we excluded donation cycles and only included single-blastocyst transfers under the age of 37 years. However, performing single-embryo transfer is the recommended practice in egg or embryo donation cycles. Therefore, we believe our findings can also be generalizable to egg or embryo donation cycle transfers.

Retrospective evaluation of our data could be accepted as the limitation of our study; therefore, prospective large randomized studies can be held for future.

Conclusion

In our study, the replacement of using G-Rinse medium solution to physiologic saline solution for ectocervical washings did not change CPR and LBR outcomes. Using physiologic saline solution can be a safe and effective alternative approach for ectocervical washing during ET in selected population to G-Rinse solution because of its lower cost, easy accessibility, and common use.

Data Availability The data in the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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