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The relationship between microbial contaminations of embryo transfer catheters with pregnancy outcome after embryo transfer

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Background: Different stages of assisted reproductive technologies are susceptible to contamination by various microorganisms.
Objective: The aim of the study was to investigate the relationship between microbial contamination of embryo transfer catheters and the pregnancy outcome after embryo transfer.
Methods: This cohort study was conducted on 60 patients candied for in vitro fertilization and embryo transfer cycles from 2021 to 2022. All embryos were transferred using a sterile syringe. The catheter contamination was checked by the microbial culture method, and in the case of microbial culture that were negative, polymerase chain reaction was done to confirm the result. The data analyzed using STATA 17 to determine the impact of catheter contamination on the clinical pregnancy rate.
Results: The average age of peoples whose microbial culture was positive was lower than that of people whose microbial culture was negative ($P < 0.05$). Also the results showed that people who live in villages have more positive microbial cultures than people who live in cities ($P < 0.05$). Also there is no difference between the number of successful implantations and the pregnancy outcome between people whose microbial culture results were positive or negative.
Conclusion: The results of the current study showed that the contamination of the embryo transfer catheter with microorganisms under our investigation did not affect the pregnancy outcome.

Keywords: embryo transfer catheters, microbial contamination, pregnancy outcome

Introduction

Different stages of assisted reproductive technologies are susceptible to contamination by various microorganisms, especially viruses. There are many effective factors in the success of In vitro fertilization (IVF) and intracytoplasmic sperm injection infertility treatment, which can be mentioned as age, thickness of the endometrium, the position of the transferred air bubbles and the duration of infertility[1–6]. Also, one of the most important factors is the laboratory conditions of the embryo contaminated[7]. In the study, Kroon and colleagues concluded that there are many factors that may affect the success of embryo transfer, including the presence of microbial colonization of the upper genital tract in women. They also suggested that prescribing antibiotics before embryo transfer, as an intervention, can reduce the rate of microbial colonization and thus improve the pregnancy rate[8]. Also, in the study of Pelzer *et al.*[9], they concluded that follicular fluid is not sterile and microorganisms colonized in the follicular fluid and the subsequent cytokine response can predict the adverse results of assisted reproductive technology and infertility. It is the cervix that prevents the ascent of the microorganism to the endometrial cavity[10]. Microbiota refers to the accumulation and colonization of microorganisms in human tissues that are exposed to the environment and external devices[11]. The bacterial composition of the vaginal microbiota is partially related to the outcome of childbirth[12]. Since in vitro fertilization treatment involves placing the embryo in the uterine cavity using a catheter that passes through the cervix, there is a clear possibility of bacterial contamination during the embryo transfer procedure. In fact, there is increasing evidence that shows that bacterial contamination of the uterine cavity after embryo transfer can negatively affect the implantation rate and pregnancy outcome. Such contamination can occur during embryo replacement through the tip of the embryo transfer catheter by various types of vaginal and

HIGHLIGHTS

- Contamination of the embryo transfer catheter is not related to the cause of infertility, but people who live in the village have a more positive microbial culture. There is no relationship between the cause of infertility and the result of microbial culture.

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uterine microorganisms. Clinical studies have shown that bacterial contamination of the embryo transfer catheter has a great negative effect on the clinical pregnancy rate[2,11]. The role of microbial infection in the outcome of IVF and embryo transfer (IVF-ET) treatment has been studied by several investigators with conflicting results. Neisseria and Chlamydia trachomatis may be seen in endocervix. These are some of the bacteria that may infect an embryo transfer catheter. Detection of Chlamydia species in the endocervix of women undergoing IVF-ET has been associated with a decrease in the rate of implantation and the rate of ongoing pregnancy. However, the identification of such microorganisms requires the use of invasive methods to collect endometrial tissue samples. Which can affect the outcome of pregnancy[13,14]. In a study conducted by Al-Rukeimi and colleagues, a significant reduction in pregnancy rates in patients whose embryo transfer catheters were infected with Pseudomonas aeruginosa and E. coli were found to be positive. They finally concluded that the bacterial colonization of the catheter tip, especially with Pseudomonas aeruginosa and E. coli was associated with a decrease in clinical pregnancy rate[15].

Despite the advances in modern infertility treatments, including the IVF method, the overall fertility rate is still low. One of the important factors in the reproductive process is the implantation of the embryo. Some researchers believe that the transfer of bacteria during the transfer of the embryo into the endometrium can have a negative effect on the process and cause a decrease in the fertility rate[16]. Because microbial contamination in embryo transfer may affect implantation rate and also due to the importance of this issue, the aim of the study was to investigate the relationship between microbial contamination of embryo transfer catheters and the pregnancy outcome after embryo transfer.

Methods

Study setting and design

In this cohort study, the 60 IVF-ET cycles conducted. All patients who came to Infertility Treatment Center within a period of one year were examined. The protocol of this study was approved by the Human Ethics Committee (RI.MUK.REC 1396/359). Written informed consent was obtained from the patient for publication and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request. Embryo transfer procedures were performed by an infertility treatment specialist. First, the vagina and cervix were cleaned with normal saline or culture medium (disinfectant solution will not be used) and the patient was prepared for embryo transfer. All embryos were transferred using a sterile syringe.

The uterine preparation protocol was performed before embryo transfer, so that patients received 6 mg of estradiol daily from the third day of menstruation, and ultrasound was performed 10 days after the start of estradiol. If the thickness of the endometrium is between 7 and 14 mm in ultrasound, embryo transfer was done. It should be noted that contact between the catheter and the vaginal wall was avoided during embryo transfer.

After each successful embryo transfer, using sterile scissors, the tip of the 2 cm catheter was inserted into the test tube containing the liquid culture medium. The presence of bacteria was checked by the microbial culture method, and in the case of microbial culture that were negative, polymerase chain reaction (PCR) was done.

Microbial culture

First, the samples were added to blood agar and McConkey media, after 24 h of incubation, the grown colonies were tested and classified into two categories: Gram-positive cocci and Gram-negative bacilli. Gram-positive cocci were initially measured with catalase test, if the catalase test was positive, coagulase test, resistance to tubiocin and urease were used to check the staphylococcus species. If the catalase test was negative, tests such as checking the type of haemolysis, sensitivity to bastracin, checking the Kemp's test, etc., were performed to check the type of streptococcus species. For gram-negative bacilli that were grown on McConkey's medium, oxidase test and then gallery diagnostic tests such as TSI, SIM, citrate, urea and phenylalanine were used to determine the bacteria in the Enterobacteriaceae family.

Polymerase chain reaction

DNA extraction was done using a DNA extraction kit (Sambayo-Thailand). The quality of the obtained product was checked using a spectrophotometer at 260 and 280 nm. Five microlitres of the obtained DNA was used as a template in a 25 µl reaction. Also, 12.5 µl of Mastermix 2X (Sambayo-Thailand), 1 µl of forward and reverse primers were used for each reaction (Table 1). Finally, the reaction volume was increased to 25 µl with distilled water. The polymerase chain reaction program for sample amplification was as follows: initial annealing temperature of 95°C for 5 min, internal annealing temperature of 95°C for 40 s, internal extension temperature of 72°C for 1 min and final extension temperature of 72°C. Also, the number of thermal cycles for reproduction was considered 35 cycles.

Statistical analysis

STATA software version 17 was used for data analysis. Qualitative variables in two groups were compared with Fisher's exact test and χ^2 test. Independent *t*-test was used to compare normal quantitative variables in two groups, and non-parametric Mann–Whitney test was used to compare non-normal quantitative variables. The assumption of normality of quantitative variables was checked using the Shapiro–Wilk test. A significance level of 5% was considered.

Results

In this study, embryo transfer was performed by an infertility and embryology specialist. Catheter tip samples were collected by a student of obstetrics and gynaecology and sent to the laboratory. Microbial culture and PCR were performed by a bacteriologist. Out of 60 samples, the microbial test results of 15 samples were positive. The results of the positive tests were that the Streptococcaceae was observed in five cases (33.33%), the Micrococcaceae was observed in three cases (20%), and the Enterobacteriaceae was observed in seven cases (46.67%) (Table 1).

The demographic information of the patients is shown in Table 2. The average age of people whose microbiological culture was positive was significantly lower than that of people whose microbiological culture was negative ($P < 0.05$). In terms of gender, no significant difference was observed between the people whose microbial culture results were positive or negative. People with a shorter period of infertility had more positive microbial

Table 1
Primers

Bacteria	Sequences	Length	Annealing temperature
Nesria Ganor A	CGGCAGCATTCAATTTGTT AAAAAGCCGCCATTTTTGTA	162	47
Chlamydia trachomatis	GGGAATCCTGCTGAACCAA TCAAAACACGGTCGAAAACA	336	47
Mycoplasma genitalium	ACCTTGATGGTCAGCAAAACTT CCTTTGATCTCATTCCAATCAGTA	193	47
Trichomonas vaginalis	ATTGTCGAACATTGGTCTTACCCTC TCTGTGCCGTCTTCAAGTATGC	263	50
Streptococcus agalactiae	CAATCCTAAGTATTTTCGGTTCATT TAGGAACATGTTCATTAACATAGC	688	47.5
Lactobacillus	CTC AAA ACT AAA CAA AGT TTC CTT GTA CAC ACC GCC CGT CA	210	47.5

culture results than those with a longer period of infertility ($P < 0.05$). There was no significant relationship between height, weight, FSH level and endometrial thickness between people who had positive or negative microbial culture results. The results showed that people who live in villages have more positive microbial cultures than people who live in cities ($P < 0.05$).

The results showed that there is no significant relationship between the cause of infertility and the result of microbial culture (Table 3).

Our results showed that there is no significant difference between the successful implantations and the pregnancy outcome between people whose microbial culture results were positive or negative. 91.11% of people who had a negative culture result had an unsuccessful pregnancy, and people who had a negative culture result had an unsuccessful pregnancy in 80% of cases (Tables 4 and 5).

Discussion

Our results has shown that the average age of peoples whose microbial culture was positive was lower than that of people whose microbial culture was negative. Also the results showed that there is no difference between the number of successful implantations and the pregnancy outcome between people whose microbial culture results were positive or negative.

All infertility treatment centres are aware of the importance of the environment of the embryo culture room, so contamination from it rarely happens. However, microbial contamination of the embryo transfer catheter is unavoidable[17]. However, there are various studies, in some of which catheter contamination is effective on the outcome of pregnancy, and in some of them it is not effective. Therefore, the type of microorganism may affect the outcome of pregnancy.

Zheng *et al.*[18], reported for the first time that embryo contamination caused by Staphylococcus pasteuri colonization does not seem to have an effect on laboratory outcome and pregnancy outcome of embryos. In Maduka and colleagues' study, 80 patients were selected and both catheter tip and cervical swabs were prepared and cultured. Patients were divided into positive and negative groups based on culture results, and at the end, clinical pregnancy rates were compared between the two groups. In 34 patients (42.25%), cervical culture was positive. Catheter tip culture was positive in 27 patients (33.75%) and negative in 53 patients (66.25%). The predominant microorganisms isolated were *Escherichia coli* (23.75%), *Staphylococcus* (18.75%) and *Streptococcus spp* (15%). Clinical pregnancy rate was 26.25%. Finally, they concluded that bacterial colonization of the catheter tip is associated with a decrease in clinical pregnancy rate[19]. In a study, 25 IVF candidate patients were examined and samples of embryo transfer catheter tips after transfer were examined for bacterial contamination. Multiple linear regression analysis showed that the presence of bacterial contamination is not significantly related to the pregnancy rate. Finally, they concluded that the presence of bacterial contamination of the catheter tip during embryo transfer is quite limited and does not significantly affect the outcome of the cycle. But they suggested that vaginal or systemic probiotics be used during embryo transfer[16]. In a prospective clinical trial study, conducted by Shehata and

Table 2
The result of bacterial culture

Bacteria	Number	%
Streptococcaceae	5	33.33
Micrococcaceae	3	20.00
Enterobacteriaceae	7	46.67

Table 3
Demographic information of patients.

	Positive culture Mean (SD)	Negative culture Mean (SD)	Statistical test	*P*
Age	31.53 (3.92)	35.31 (5.43)	Independent *t*-test	0.01
Infertile man	4 (30.77)	8 (21.62)	Fisher's exact test	
Infertile women	8 (61.54)	24 (64.86)	Fisher's exact test	
Infertility in both sexes	1 (7.69)	5 (13.51)	Fisher's exact test	0.79
Infertility period	7.93 (3.95)	9.66 (5.92)	Independent *t*-test	0.03
Weight	76.8 (12.82)	73.62 (9.26)	Independent *t*-test	0.30
Height	162.73 (8.48)	162.33 (5.60)	Independent *t*-test	0.83
FSH	5.70 (4.45)	6.48 (3.43)	Mann–Whitney test	0.22
Endometrial thickness	8.90 (1.37)	9.56 (1.63)	Mann–Whitney test	0.17
	N (%)	*N* (%)		
Urban	10 (66.67)	39 (86.67)	χ^2 test	
Rural	5 (33.33)	6 (13.33)	χ^2 test	0.08
Embryo grade			χ^2 test	
A	6 (40.00)	20 (44.40)		
A + B	6 (40.00)	17 (37.80)		
B	3 (20.00)	8 (17.80)		1.00

FSH indicates follicle-stimulating hormone.

Table 4

The cause of infertility in patients.

	Positive culture	Negative culture	Statistical test	*P*
	N (%)	*N* (%)	Fisher's exact test	0.78
PCOS	9 (60.00)	21 (46.67)		
Oligospermia	2 (13.33)	6 (13.33)		
Azoospermia	2 (13.33)	3 (6.67)		
Diminished ovarian reserve	0	3 (6.67)		
Vaginismus and old age of women	0	1 (2.22)		
Azoospermia and PCOS	1 (6.67)	1 (2.22)		
Hyperprolactinemia	0	2 (4.44)		
Asthenospermia and PCOS	1 (6.67)	1 (2.22)		
Oligospermia and Diminished ovarian reserve	0	1 (2.22)		
Oligospermia and PCOS	0	5 (11.11)		
Varicocele and old age of women	0	1 (2.22)		

PCOS indicates polycystic ovary syndrome.

colleagues in 2012–2013, 81 IVF candidate patients were examined. Catheter tip culture was mainly positive for *Escherichia coli* (64%) and *Streptococcus* species (80%). Finally, they concluded that the presence of bacterial contamination of the catheter tip during embryo transfer does not significantly affect cycle results, which may be due to the effect of mucus in the cervical canal that prevents microorganisms from climbing into the endometrial cavity[20].

In a study conducted by Al-Rukeimi and colleagues, in 2016–2017, 162 patients aged 23–38 years, who were undergoing IVF treatment, were selected for this study. The pregnancy rate was not significantly different between positive and negative bacterial growth, but there was a significant decrease in pregnancy in patients whose embryo transfer catheter was Pseudomonas aeruginosa and *E. coli* were found to be positive. They finally concluded that bacterial colonization at the catheter tip, especially with *Pseudomonas aeruginosa* and *E. coli* is associated with a decrease in clinical pregnancy rate[21]. The strength of this study was the assessment of catheter contamination with microbial culture and PCR confirmation, and the results were interpreted based on the type of bacteria. However, conducting more studies with a larger statistical population is suggested.

Table 5

Pregnancy outcome in the studied groups

	Positive culture	Negative culture	Statistical test	*P*
	N (%)	*N* (%)	Fisher's exact test	0.34
Successful implantation	3 (20.00)	4 (8.89)		
Pregnancy leads to birth	3 (20.00)	4 (8.89)		
Unsuccessful implantation	12 (80.00)	41 (91.11)		
Failed pregnancy	12 (80.00)	41 (91.11)		

Conclusion

In the current study, the results of the positive tests were that the Streptococcaceae was observed in 33.33%, the Micrococcaceae was observed in 20%, and the Enterobacteriaceae was observed in 46.67%. Therefore, most cases of positive embryo transfer catheter contamination were related to Enterobacteriaceae family bacteria. However, there was no significant relationship between the type of bacteria and the outcome of unsuccessful pregnancy. The results of the current study showed that the contamination of the embryo transfer catheter with microorganisms under our investigation did not affect the pregnancy outcome.

Ethical approval

Ethical approval for this study (Number: RI.MUK.REH1396/359) was provided by the Ethical Committee of the Kurdistan University of Medical Sciences, Iran on 8 June 2021.

Consent

Consent is available for review by the Editor-in-Chief of this journal on request.

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Kurdistan University of Medical Sciences, Sanandaj, Iran.

Author contribution

All authors contributed equally to this article.

Conflicts of interest disclosure

There are no conflicts of interest.

Research registration unique identifying number (UIN)

The site http://www.researchregistry.com is down and registration is not possible. We have done this based on the approvals of the ethics committee of the Kurdistan University of Medical Sciences. It should be noted that our intervention was not on humans, and we only sampled the catheter tip, which had to be discarded. However, all information is kept confidential.

Guarantor

Masoumeh Rezaie is the person in charge of the publication of our manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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