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The levels of bacterial contamination of the embryo transfer catheter relate negatively to the outcome of embryo transfer

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ABSTRACT

Objective: To determine the prognostic value of presence and level of bacterial contamination on the pregnancy outcome in patients undergoing embryo transfer following IVF/ICSI.

Design: Prospective, cross-sectional observational study

Materials and methods: Twenty-five consecutive patients undergoing an embryo transfer cycle as a part of their IVF/ ICSI treatment were included. Cervical mucus samples were taken immediately prior to embryo transfer and the tips of the post-transfer catheters were examined for bacterial contamination, and their levels were recorded.

Results: The presences of bacterial contamination in the cervical and catheter tip samples showed a weak correlation between finding gram positive and gram negative bacteria on the cervical sample and the catheter tip. Multiple linear regression analysis demonstrated that the presence of bacterial contamination was not significantly associated with the pregnancy rate.

Conclusion(s): The presence of bacterial contamination of catheter tips during embryo transfer is evidently limited, and does not significantly affect the cycle outcomes.

Key words: Bacterial contamination, Vaginal flora, Embryo transfer, Assisted Reproduction

Assisted reproductive techniques (ARTs), such as transvaginal oocyte pick-up and catheter insertion for intrauterine insemination or embryo transfer, are considered to be relatively safe procedures (1). In general, acute pelvic infection following ARTs is generally uncommon despite the invasive nature of such procedures (2-3). Any risk of infection arises from the transfer of micro-organisms that make up the normal vaginal flora to the uterine cavity and pelvic peritoneum.

The fear that genital bacterial contamination may interfere with embryo implantation has been suggested as far back as 1978 (4). Clinical studies have shown that bacterial contamination of the embryo transfer catheter has a significant negative effect on the clinical pregnancy rates (5 – 8).

In addition, cervical sterility at the time of ART procedures cannot be achieved with the routine use of vaginal antiseptics since there is evidence these solutions have been shown to have a negative impact on the quality of the oocytes collected and the embryos available for transfer (9). Moreover, there is insufficient evidence about the effects of different antibiotic prophylaxis regimens on ART cycle outcomes (8, 10 – 12).

The purpose of this study was to evaluate the rate of bacterial contamination of cervical mucus samples from women at the time of embryo

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transfer. In addition, the rate of bacterial contamination of catheters following embryo transfer, as well as the outcomes of ART cycles with regards different levels of contamination was determined.

MATERIALS AND METHODS

Participants

Twenty-five consecutive patients undergoing an embryo transfer as part of their IVF/ ICSI treatment were screened and included. The inclusion criteria consisted of female partners of infertile couples, aged 18 – 39 years old. Exclusion criteria were concurrent use of antibiotics, and/ or other indication for antibiotic therapy, patients with a history of previous pelvic infection and/ or high risk of pelvic infection that require i.v. antibiotic prophylaxis, patient age >39 years at time of embryo transfer, any significant cardiovascular, pulmonary, neurological, allergic, hepatic or renal disease. Each woman participated only once in the study. Ethical committee approval was obtained and all patients gave their oral consent to be included in the study.

The patients and the embryologist were blinded to the results of the bacteriologic examination. The microbiologist was blinded to the results of the treatment cycle.

Culture samples

Cervical sample (pre-transfer)

A sterile cusco speculum was inserted into the vagina and opened so that the external os is visualized. Using a sterile insulin syringe, the cervical mucus and secretions were aspirated. The aspirated secretions were placed onto a 5% sheep blood agar plate. The plates were incubated aerobically and under 5% CO2 at 37°C for 72h.

Catheter tip sample (post-transfer)

The vagina and cervix were cleaned with normal saline or culture media (no antiseptic solution was used) and the patient prepared for the embryo transfer. Any apparent vaginal and cervical secretions were removed using sterile cotton swabs. All embryos were transferred using the same catheter type, using a non-touch sterile replacement technique (sterile drapes, speculum and disposable non-latex gloves). Contact between the transfer catheter, the vaginal walls and ecto-cervix was avoided.

Following withdrawal of the catheter, and confirmation that the embryos had been transferred, the embryologist cut off the distal 2 cm of the catheter tip using sterile scissors. Each individual tip was rolled on to a 5% sheep blood agar plate, using sterile forceps. The plates were incubated aerobically and under 5% CO2at 37°C for 72h.

Microbiological assessment

The plates and broth solution were incubated aerobically at 37°C for 48h. Following the incubation period, a single microbiologist, blinded to the randomization allocation, performed the microbiological assessment of the plates. Bacteria were identified by standard laboratory techniques (13) and quantified using a semi-quantitative four-point grading system for gram-positive and gram-negative organisms: the absence of growth after 48 h [no growth (NG)], <10 bacterial colonies (+), >10 bacterial colonies (++) and semi-confluent or confluent growth (+++) on the blood agar plate. Positive Brain Heart Infusion (BHI) cultures were plated out, and the bacteria identified then graded as (+) (14).

RESULTS

Twenty five women undergoing embryo transfer were included in the analysis. The organisms identified are illustrated in Table 1. The presence of bacterial contamination in the cervical and catheter tip samples was correlated against each other and against the incidence of a clinical pregnancy. There was a weak correlation between the presence of gram positive bacteria on the cervical sample and the catheter tip (Correlation coefficient (r) = 0.10 (r^2= 0.009), 95% CI for r = -0.31 to 0.47, P = 0.65). This was also similar for

Table 1. Type of organisms identified in patients who underwent embryo transfer.

	Cervical sample	Catheter sample
Gram positive bacteria	21 (84.00%)	14 (56.00%)
Staphylococcus Aureus	2 (8.00%)	3 (12.00%)
Coagulase-negative Staphyloccus (CoNS)	8 (32.00%)	7 (28.00%)
Streptococci	10 (40.00%)	5 (20.00%)
Diphteroids	1 (4.00%)	1 (4.00%)
Lactobacilli	18 (72.00%)	6 (24.00%)
Gram negative bacteria	4 (16.00%)	3 (12.00%)
Klebsilla spp.	2 (8.00%)	0 (0.00%)
Psuedomonous spp.	2 (8.00%)	0 (0.00%)
Proteus	0 (0.00%)	1 (4.00%)
Non-lactose fermenters (NLF)	0 (0.00%)	2 (8.00%)
Escherichia coli (E.Coli)	1 (4.00%)	0 (0.00%)

gram negative bacteria (Correlation coefficient (r) = -0.16 (r^2= 0.03, 95% CI for r = -0.52 to 0.25, P = 0.44).

As for the presence of bacterial contamination on the embryo implantation rate, multiple linear regression demonstrated that the presence of bacterial contamination was not significantly associated with the pregnancy rate (Table 2). This was also confirmed by simple linear regression analyses (Table 3).

In addition, the level of bacterial contamination on the cervical mucus and catheter tip samples were correlated against each other, and against the incidence of a clinical pregnancy. There was a weak correlation between finding gram positive bacteria on the cervical sample and the catheter tip (Correlation coefficient (r) = 0.21 (r^2= 0.05), 95% CI for r = -0.20 to 0.56, P = 0.31). This was also similar for gram negative bacteria (Correlation coefficient (r) = -0.11 (r^2= 0.01), 95% CI for r = -0.49 to 0.30, P = 0.60).

Moreover, regarding the effect of bacterial contamination on the embryo implantation rate, multiple linear regression demonstrated that the level of bacterial contamination with gram positive bacteria was the only factor that was significantly associated with the pregnancy rate (Table 2). This was confirmed by simple linear regression analyses on the level of contamination with gram positive and negative bacteria in the cervical mucus and catheter tip samples (Table 3).

DISCUSSION

The bacterial flora of the female reproductive tract is a focal point for the study of infectious disease in obstetrics and gynecology, as it is recognized that many pelvic infections involve bacteria resident on the cervical-vaginal epithelium. The vaginal flora contains a large variety of bacterial species, including aerobic and anaerobic organisms, as revealed by modern microbiologic methods (15). Moreover, the diversity and kinds of organisms that comprise the vaginal microbial community vary among women (16).

Since the lower genital tract is a naturally inhabited with vaginal flora and pathogenic organisms, operative procedures through or adjacent to this field leads to a moderate to high incidence of infection. Therefore recommendations for antibiotic prophylaxis have been established in many procedures, including vaginal hysterectomy, abdominal hysterectomy, and cesarean section (17). However, unlike most assisted reproductive techniques, these are major operative procedures that may carry a high morbidity rate from infections.

With regards minor operative procedures related to ART, such as during trans-vaginal oocyte retrieval and embryo transfer, there are no clear recommendations by any society (e.g. American society of Reproductive Medicine [ASRM], European Society for Human Reproduction and Embryology [ESHRE], Middle East Fertility Society [MEFS], Mediterranean Society for Reproductive Medicine [MSRM]) or evidence-based guidelines (e.g. NICE guidelines).

Table 2. Multiple linear regression analysis correlating bacterial presence and levels with the incidence of a clinical pregnancy.

Bacterial presence	Gram +ve in cervix	P = 0.71
	Gram -ve bacteria in cervix	P = 0.54
	Gram +ve bacteria on catheter tip	P = 0.15
	Gram -ve bacteria on catheter tip	P = 0.72
Bacterial levels	Gram +ve in cervix	P = 0.47
	Gram -ve bacteria in cervix	P = 0.31
	Gram +ve bacteria on catheter tip	P = 0.006
	Gram -ve bacteria on catheter tip	P = 0.69

Table 3. Simple linear regression analyses correlating bacterial levels with the incidence of a clinical pregnancy.

Bacterial presence	Gram +ve in cervix	Correlation coefficient (r) = -0.02 (r^2= 0.0003), 95% CI for r = -0.41 to 0.38, P = 0.94
	Gram -ve bacteria in cervix	Correlation coefficient (r) = 0.02 (r^2= 0.0003), 95% CI for r = -0.38 to 0.41, P = 0.94
	Gram +ve bacteria on catheter tip	Correlation coefficient (r) = 0.30 (r^2= 0.09), 95% CI for r = -0.11 to 0.62, P = 0.15
	Gram -ve bacteria on catheter tip	Correlation coefficient (r) = -0.11 (r^2= 0.01), 95% CI for r = -0.48 to 0.30, P = 0.61
Bacterial levels	Gram +ve in cervix	Correlation coefficient (r) = 0.29 (r^2= 0.08), 95% CI for r = -0.12 to 0.61, P = 0.16
	Gram -ve bacteria in cervix	Correlation coefficient (r) = 0.18 (r^2= 0.03), 95% CI for r = -0.23 to 0.54, P = 0.39
	Gram +ve bacteria on catheter tip	Correlation coefficient (r) = 0.58 (r^2= 0.34), 95% CI for r = 0.24 to 0.80, P = 0.002
	Gram -ve bacteria on catheter tip	Correlation coefficient (r) = -0.11 (r^2= 0.01), 95% CI for r = -0.48 to 0.30, P = 0.61

However, these procedures have a high possibility of ascending infection from the lower genital tract to the upper genital tract, especially for those procedures that pass through the endocervical canal into the uterine cavity (e.g. intrauterine insemination and embryo transfer). Since these procedures have only small areas of tissue trauma, it is questionable whether or not antibiotic prophylaxis, the use of antibiotics for the prevention of infection, for these procedures protects against ascending infection. Therefore, antibiotic prophylaxis might have a role to prevent infection in these procedures, but this has yet to be officially quantified.

In essence, in today's evidence based medical environment, any recommendation must be built on two main questions: (1) whether ascending infections occur as a result of the procedure and (2) whether this results in a decreased pregnancy rate in such cases. Only then can a proper set of guidelines be proposed to answer this clinical query.

The results of this prospective cohort demonstrated that there is limited evidence between the presence of bacterial colonies in the cervical mucus samples and the tips of embryo transfer catheters. In addition, there was an observed negative relationship between the presence of gram positive bacterial colonies and embryo implantation following embryo transfer. Even so, this trend was not observed with the presence of gram negative bacterial colonies on the catheter tips, nor gram positive or negative bacterial colonies in the cervical mucus samples. Therefore, in accordance with the results of this study, it is recommended that if antibiotic prophylaxis is used during embryo transfer, it should mainly cover gram positive bacteria.

A recent randomized controlled trial of co-amoxicillin versus placebo showed that bacterial contamination of the transfer catheter during embryo transfer is associated with poor clinical outcomes (14). In addition, they demonstrated that antibiotics significantly reduced catheter contamination rates (49.4 versus 62.3%, RR = 0.79, 95% CI: 0.64, 0.97, P = 0.03), but there was no difference detected in clinical pregnancy rates between the two groups (36.0 versus 35.5%, P = 0.83). Even so, there was a significant (P = 0.03) association between the level of bacterial contamination and clinical pregnancy rates.

In conclusion, the results of this prospective cohort demonstrate that the presence of bacterial contamination of catheter tips during embryo transfer is evidently limited, and does not significantly affect the cycle outcomes. This trend is in accordance with the available literature on the use of vaginal or systemic probiotics during embryo transfer.

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