

An Observational Study of IVF Outcomes with The Inference of Bacterial Vaginosis (BV) Infection

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ARTICLE INFO

Article History:

Accepted: 05 April 2023 Published: 30 April 2023

Publication Issue

Volume 10, Issue 2 March-April-2023

Page Number

943-947

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ABSTRACT

Objective: The purpose of this study is to investigate the effect of bacterial vaginosis on females who are undergoing IVF treatment. Background: It is a fascinating process that enables infertile couples who are having difficulty conceiving naturally to achieve pregnancy through ART. The most common procedure in ART is IVF, where embryo transfer is one of the crucial steps in achieving a successful pregnancy. IVF is a complex series of procedures used to enhance fertility, address genetic problems, and aid in the conception of a child. During IVF, mature eggs are retrieved from the ovaries and fertilized with sperm, and further culture the embryos in an IVF laboratory. In assisted reproduction, embryos are transferred into a female's uterus in order to establish a pregnancy. It should be noted that there is still a wide range of indications and associated risks that affect embryo transfer procedures. Bacterial infection is one of the most common complications. Infection due to bacteria in the vagina known as bacterial vaginosis occurs when some normal microflora grows too large, resulting in bacterial imbalances. Symptoms include off-white or grey vaginal discharge that smells fishy. Increasing evidence suggests that bacterial vaginosis may not only cause disease in the lower genital tract. In-vitro fertilization (IVF) patients were examined for potential associations with infertility and effects on fertilization and implantation. Methodology: A total of 135 infertile couples were counseled for this study, however only 100 agreed to participate. In this observational study, we examined the effect of bacterial infection on IVF outcomes among infertile females (n=100) receiving IVF treatment at Aashakiran IVF, Kharar, Punjab, between July 2020 to December 2022. Informed consent

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was obtained from all patients prior to the procedure. For all females in this study, unexplained categories of patients were enrolled who had normal tubal patency, regular ovulation, and no male factors. A swab sample (n=100) was taken from the patient before embryo transfer. There are still many indications and associated risks that affect the embryo transfer procedure. The sample was cultured on a blood agar plate and isolated from the sample for up to 48 hours. They first checked after 24 hours, if the growth was not present then incubated for up to 48 hours. Then we compared their IVF outcomes in the samples in which we found bacterial growth. Result: 34 samples with bacterial growth showed 15(44.11%) patients get positive IVF results and 19 (55.88%) patients get negative IVF results. Whereas overall 66 samples without any bacterial growth showed 32 (48.48%) positive IVF results and 34 (51.51%) negative IVF outcomes out of 100 patients. The difference in IVF outcome between bacterial growth and no growth of BV is 4.37% only. Thus, this study does not support the hypothesis that BV adversely affects embryo implantation and outcome. Conclusion: The present study concluded that somewhere BV affects IVF outcomes. For more understanding of BV infection how much effect IVF results then need to study higher patient data.

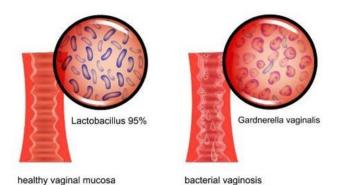
Keywords: IVF, ART, Bacterial Vaginosis, swab, isolation, and ET

I. INTRODUCTION

As one of the most common vaginal infections, Bacterial Vaginosis (BV) affects the vaginal flora, causing pathogenic effects as well as increasing the risk of miscarriage in women who are pregnant. Santa María-Ortiz Johana K, et. al., 2022, shows that lactobacilli are the most important bacteria responsible for maintaining the microflora in the vaginal environment. Furthermore, it maintains the natural balance of the vagina and prevents genital infection. When there is a change in vaginal flora, it can alter the pH of the acidic environment, resulting in genital disorders. In vitro, fertilization patients with altered vaginal microbiota have been reported to have poor reproductive outcomes. In a study by van Oostrum N (Van Oostrum, N, et. al., 2013), 19% of the infertile population has a prevalence of bacterial vaginosis. A

few studies have shown a contradictory relationship between abnormal vaginal flora and birth rates in reproductive outcomes (D E Moore, *et al.*, 2000). Furthermore, studies suggest that BV in the vaginal environment does not affect the birth rate of women with high levels of lactobacillus in the vaginal flora. In addition to lactobacillus, other anaerobic bacteria such as Gardnerella vaginalis, mycoplasma hominis, atopobium vaginae, and mobiluncus curtisii maintain the vaginal environment to prevent infection and maintain the vaginal flora.

BACTERIAL VAGINOSIS



As reported in a study (J Mangot-Bertrand, et, al., 2013), bacterial vaginosis affects embryo implantation, miscarriages, early spontaneous and negative pregnancy outcomes for infertile women. The patient completed a short questionnaire on the day of embryo transfer regarding factors for which we were unable to control and which were thought likely to influence vaginal bacterial flora. These factors included antibiotics administration, duration of infertility, cause of infertility, sexual history, personal hygiene, pubic hair, intimate hygiene, contraception history, pelvic inflammatory disease, and STDs.

II. METHODS AND MATERIAL

The objective of this observational study is to analyze bacteria samples collected from 100 infertile females undergoing IVF treatment with Aashakiran IVF. Each of the females had primary or secondary infertility for a period of more than two years. The term unexplained infertility refers to couples who are experiencing infertility despite having normal tubal patency, regular ovulation, and no cervical factors which regularly trigger unprotected intercourse over a period of at least one year.

Females undergoing treatment where urinary folliclestimulating hormone was the primary method of ovarian stimulation. Vaginal ultrasonography and measurement of serum estrogen were used to monitor the development of ovarian follicles. 36 hours after administering 5000 IU of human chorionic gonadotrophin, oocytes were collected via vaginal ultrasound-guided collection. Two additional injections of 2000 IU of HCG, two and seven days after the collection of the oocytes, provided luteal support. Two pronuclei and progressive cleavage up until the time of embryo transfer defined normal fertilization. Three embryos were maximum transferred to the uterus following IVF on the third day of embryo culture. After four weeks, transvaginal ultrasound evidence of a gestation sac confirmed the pregnancy. The total number of gestational sacs as a percentage of the total number of transferred embryos was used to calculate the implantation rate for each patient group studied. Miscarriage and birth rates, as well as babies as a percentage of transferred embryos, were calculated for each pregnancy.

Before embryo transfer a swab was obtained from a patient receiving fertility treatment to be analyzed for bacterial vaginosis. A total of 100 samples were processed from the vagina of different patients and analyzed for bacterial growth by using a blood agar plate. A sterile vaginal speculum was passed and a sterile cotton swab stick was used to obtain a sample of posterior vaginal fluid. The speculum was inserted into the vagina and was to open until the cervix of the patient become visible; the swab stick was then used to collect the sample at the site below the cervix by gently rotating it around the site under the supervision of the gynecologist.

Here, blood agar was to isolate the growth of bacteria in petri plates kept for incubation. Contents for blood agar are sodium chloride, sheep blood, protein source, soybean protein digest, and agar.

Preparation of Blood Agar:

- Blood agar was prepared in a flask by dissolving 11 gm of powdered agar media in 500 ml of distilled water.
- The flask was shaken for some time to dissolve the medium completely and then sterilized by using an autoclave at 121 Degree Celsius temperature for 15 minutes.
- Pour that sterile media onto the petri dish and



allow it to cool and solidify.

- Streak the swab on the blood agar plate and kept it in the incubator at 37°C for 24 hours.
- Checked for Petri dishes with no bacterial growth and incubate them for a further 48 hours (Carola P., *et al*, 2015).

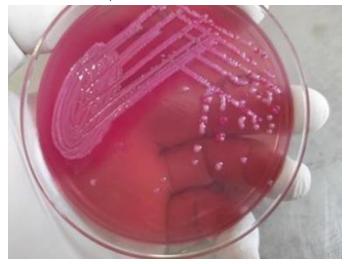


Fig 1 : Petri dish with bacterial growth Then Observed the result of IVF outcomes in which bacterial growth was seen, so that it can be known whether bacterial vaginosis affects IVF outcomes: Table 1

Table 1: Showing the IVF outcome in those patients who have an infection with bacterial vaginosis or no infections with BV:

TOTAL SWAB SAMPLES (n=100)			
Bacterial Growth		No bacterial Growth	
34		66	
IVF Outcome		IVF Outcome	
Positive	Negative	Positive	Negative
15 (44.11%)	19 (55.88%)	32 (48.48%)	34 (51.51%)

III. RESULTS AND DISCUSSION

In relation to the grades of vaginal bacterial flora, women's ages and methods of insemination had similar frequency distributions. However, BV is closely related to infertility, even though it significantly impacts conception in women who are pregnant. Furthermore, females with a lower or imbalanced vaginal bacterial concentration showed a higher incidence of embryo implantation than those with a higher prevalence of lactobacilli in their vaginal flora. (Koumans, EH, et al., 2007). A total of 100 patients were approached, consented to, and included in this study. In this study (Table 1), the prevalence of bacterial vaginosis growth group was (n=34, 34%) which showed positive IVF results in patients (n=15, 44.11%) and Negative IVF Results in patients (n=19, 55.88 %) who attended IVF treatment and Embryo Transfer (ET). Moreover, no bacterial growth group (n=66, 66%) showed positive IVF results in patients (n=32, 48.48%) and Negative IVF Results in patients (n=34, 51.51 %) who attended IVF treatment and ET. The difference in IVF outcome between bacterial growth and no BV growth is only 4.37%. Accordingly, this study does not support the hypothesis that BV adversely affects embryo implantation and outcome. In addition, bacterial vaginosis is closely associated with pelvic inflammatory disease (Bibi A, et al., 2014). However, the natural history of bacterial vaginosis remains unclear. For a more accurate and robust conclusion, it would be ideal if the study were a randomized controlled trial. Longitudinal studies are needed to conclusively determine the link between bacterial vaginosis and outcomes. The pregnancy rates differed between positive and negative bacterial growth, with a notable reduction in the pregnancy rate among those who were positive (Coleman JS, et al., 1983).

IV.CONCLUSION

According to the results, IVF's positive outcomes were lower than BV's negative outcomes. According to this study, BV significantly affects the reproductive outcomes of IVF, whether it is for rates of biochemical or clinical pregnancy. (Hill GB, 1993). We are conducting this research in order to improve the vaginal flora of infertile patients as well as the success rate of IVF. It is suggested that regular cervical swabs, microscopic cultures, and patient preparation for IVF treatment can be helpful. There was a significant increase in fertilization rates among women with



bacterial vaginosis. There may, however, be some other medical anomalies that account for a significantly greater percentage of cases than other causes of infertility, where fertilization rates are more likely to be favourable. This study challenges the hypothesis that bacterial vaginosis inhibits fertilization and implantation during IVF treatment. There was no significant difference in fertilization or implantation rates among IVF patients and antenatal or general gynecological populations despite a significantly higher prevalence of bacterial vaginosis among IVF patients than in antenatal and general gynecological populations. This means that, even though routine screening and treatment for bacterial vaginosis prior to IVF treatment would appear to be effective, there is still a possibility that treatment could reduce the risk of complications during pregnancy. Those patients who undergo IVF for tubal infertility should be considered for antenatal screening if the treatment is found to be beneficial. Ultimately, we recommended that all patients receiving infertility treatment should undergo BV testing in order to improve the success of IVF and to give positive responses to the patient.

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Cite this article as :

Harjeet Kaur, Naresh Kumar, Ram Dayal, Asmita Mahla Bhambri, "An Observational Study of IVF Outcomes with The Inference of Bacterial Vaginosis (BV) Infection ", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN: 2395-602X, Print ISSN: 2395-6011, Volume 10 Issue 2, pp. 943-947, March-April 2023. Available at doi: https://doi.org/10.32628/IJSRST523102151 Journal URL: https://ijsrst.com/IJSRST523102151