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The Prevalence of Bacterial Vaginosis among Infertile Iraqi Women and Subsequent ICSI Outcome.

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ABSTRACT

The vaginal microbial makeup has a substantial impact on women's health. The typical vaginal microbial profile in healthy women of reproductive age comprises aerobic, facultatively anaerobic, as well as obligately anaerobic organisms. However, the optimal vaginal microbiota is dominated by lactobacilli and is thought to defend against pathogenic microbial colonization and infection by producing lactic acid, antimicrobial metabolites, and lowering immune system activation. Recent research has come up with the term "molecular bacterial vaginosis (BV)" to describe non-optimal vaginal microbiota that is low in Lactobacillus species (spp.) and has a lot of facultative and absolute anaerobic bacteria specifically Gardnerella vaginalis. In the context of IVF, BV has been linked to infertility. The study comprised forty-six women to explore the vaginal and follicular microbiota of asymptomatic infertile Iraqi women starting an ICSI trial. At the time of ova pick-up, a high vaginal swab and follicular fluid were acquired from each instance for DNA extraction to test for the presence of microorganisms by the use of a real-time PCR special commercial kit. In conclusion, molecular technologies have offered a more thorough view into the vaginal microbiota, which may explain why vaginal dysbiosis shifts during the ICSI-ET treatment. Even though the majority of women have no clinically visible infection, the fact is that they have an aberrant vaginal micro-ecology profile. Nevertheless, isolating microorganisms from follicular fluid did not reduce the likelihood of fertilization or the pregnancy rate during ICSI cycles.

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KEYWORD

Bacterial vaginosis; Vaginal microbiota; Gardnerella vaginalis; ICSI

1. Introduction

The typical vaginal microbial profile in healthy women of reproductive age comprises aerobic, facultatively anaerobic, as well as obligately anaerobic organisms. However, the optimal vaginal microbiota is dominated by lactobacilli and is thought to defend against pathogenic microbial colonization and infection by producing lactic acid, antimicrobial metabolites, and lowering immune system activation **(Ravel et al., 2021 [1])**. The most prevalent cause of vaginal discharge is bacterial vaginosis (BV). The most common sign and symptom is a thin, white, "fishy"-smelling vaginal discharge, with or without itching. However, up to 50% of women may not have any signs or symptoms **(Sherrard et al., 2018 [2])**. It is identified by the loss of healthy Lactobacillus species that produce hydrogen peroxide and lactic acid as well as significant populations of facultative and entirely anaerobic bacteria **(Muzny et al., 2019 [3])**, specifically Gardnerella vaginalis (GV) **(Kunze and Larsen, 2019 [4])**, with an elevated pH**(Aldunate et al., 2015 [5])**. As a result, what was formerly thought to be a rather simple microbial population has now been proven to be dynamic and sophisticated. Each woman has a unique vaginal microbial composition that changes over time and is influenced by factors such as nutrition, lifestyle, hormonal profile, genetic factors, and age **(Gupta et al., 2019 [6])**. The formation of a polymicrobial biofilm on the surface epithelial cells of the vagina is a distinguishing hallmark of BV. The fact that there is an oxygen gradient inside the biofilm makes the presence of this biofilm a favorable habitat for anaerobic bacteria to live **(Beebout et al., 2019 [7])**.

Epidemiological evidence strongly suggests that BV is a sexually transmitted disease with a 4–day incubation period, comparable to other

bacterial agents of sexually transmitted diseases, such as Neisseria gonorrhoeae **(Muzny et al., 2019 [8])**. Recent research has come up with the term "molecular BV" to describe non-optimal vaginal microbiota that is low in Lactobacillus spp. and has a lot of facultative and absolute anaerobic bacteria. This can be found by using shotgun metagenomics sequence analysis, quantitative PCR, or metaproteomic research **(McKinnon et al., 2019 [9])**. In the context of IVF, BV has been linked to infertility. Women with less microbial diversity and a larger proportion of aberrant vaginal microbiota had worse reproductive outcomes after IVF **(Haahr et al., 2019 [10]).**

2. Patients and Methods

Serono, The current research has been conducted at Al Nahrain University's Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies in Baghdad, Iraq, for the period between November 2020 and November 2021. It has been approved by the local medical ethics committee, and each woman signed a written informed consent before being enrolled in the study. The study comprised forty-six women aged between 21 and 47 years. They were stimulated with an antagonist regimen and had no vaginal infection with fresh embryo transfers. Women who had used broad-spectrum antibiotics or local vaginal therapies before starting controlled ovarian hyperstimulation procedures or who had abnormal vaginal discharges were not included in the study.

Basic steps in the ICSI treatment cycle

Based on ovarian response as determined by hormone serum levels and ultrasound examination, FSH (150–300 IU) was used to stimulate multiple follicle development (Gonal FR, Merk, Switzerland). Once the leading follicle had reached 14 mm in diameter, a GnRH antagonist (Cetrorelix acetate for injection, 0.25

mg: Cetrotide®, Merk, Switzerland) was given daily. When at least three follicles larger than 16 mm in diameter were detected, 10,000 IU of human chorionic gonadotrophins (hCG) were injected intramuscularly (Pregnyl®, Organon, Holland), and oocyte harvest was scheduled for 34–36 hours later. To fertilize the oocytes, intracytoplasmic sperm injection was used, and embryo transfer (ET) occurred 3–5 days later. Serum hCG levels were tested 14 days after the ET. At weeks 6-7 of pregnancy, a transvaginal ultrasound was done to see if there was a gestational sac.

Collection of samples:

At the time of ova pick-up, a high vaginal swab was acquired from each instance for DNA extraction, and after the oocytes were harvested, follicular fluid was obtained for DNA extraction to test for the presence of microorganisms. The vaginal PH was determined using Henso Medical's color-fixed indicator strips (URS-3) (China).

With a commercial kit, real-time PCR was used to look for genital tract infections that have strict growth requirements or can't be grown in a lab, such as Chlamydia trachomatis, Neisseria gonorrhea, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Mycoplasma genitalium, Trichomonas vaginalis, Gardnerella vaginalis and herpes simplex virus types 1 & 2.

Statistical Analysis

The twenty-third version of the Statistical Package for Social Sciences (SPSS) and the 2019 Microsoft Office Excel were applied to gather, summarize, analyze, and represent data. Qualitative variables were articulated in numbers and proportions, and a comparison between pregnant and non-pregnant ladies was carried out by Fisher's exact test. The Shapiro-Wilk test was used to assess quantitative variables, normally distributed quantitative data were represented as mean, and standard deviation, and comparisons between

pregnant and non-pregnant women were made using an unpaired t-test, while non-normally distributed numeric variables were compared using the Mann-Whitney test. The positive findings were considered to have a P-value of less than 0.05.

3. Results

Table 1 displays the clinical features of the forty-six infertile women who took part in the current investigation and were split into two groups: pregnant (n = 13) and non-pregnant (n = 33) based on ICSI results. Patients ranged in age from 21–47 years, with no significant variance in the mean age between the non-pregnant and pregnant groups (P = 0.130). Furthermore, there was no statistically significant difference in the mean BMI between both the non-pregnant and pregnant women (P = 0.972). The mean duration of infertility extends from 1– to 17 years, with no significant difference between non-pregnant and pregnant women (P = 0.576). Moreover, the frequency distribution of infertile women displayed no significant difference according to the type of infertility, whether primary or secondary (P = 0.724).

The study showed that the mean length of stimulation varied from 6–12 days, with no significant difference between non-pregnant and pregnant women (P = 0.346). While There was no statistically significant difference in mean serum estrogen levels between non-pregnant and pregnant women on the day of HCG administration (P = 0.294).

On the day of ova collection, vaginal PH values ranged from 5 to 6.5. There was a significant difference (p = 0.001) between the mean values of the two groups, with the non-pregnant women having a higher mean vaginal PH than the pregnant women.

Table (1): Clinical characteristics of infertile women involved in the present study

Characteristic	Total number of cases (46)	Not pregnant (33)	Pregnant (13)	P-value
Age (yr.)				
Mean±SD	32.17±6.95	33.15±7.08	29.69±6.2	0.130a
Median (Range)	32 (21-47)	33 (22-47)	32 (21-41)	
BMI (kg/m2)				
Mean±SD	28.16±4.32	28.17±3.37	28.12±6.3	0.972 a
Median (Range)	27.55 (19.5-44)	28.1 (19.5-33.3)	26.3 (22-44)	
Duration of infertility (yr.)				
Mean±SD	7.2±4.35	7.42±4.45	6.62±4.21	0.576 a
Median (Range)	6 (1-17)	7 (1-16)	6 (2-17)	
Type of Infertility				
Primary, No. (%)	32 (69.6%)	22 (66.7%)	10 (76.9%)	0.724 b
Secondary, No. (%)	14 (30.4%)	11 (33.3%)	3 (23.1%)	
Duration of stimulation (day)				
Mean±SD	8.96±1.51	9.1±1.43	8.62±1.85	0.346a
Median (Range)	9 (6-12)	9 (6-12)	9 (6-12)	
E2 at Day of HCG (pg/ml)				
Mean±SD	1322.1±772.15	1227.26±718.06	1562.85±879.4	0.294*
Median (Range)	1210 (350-3000)	1292 (350-3000)	1200 (488.9-2847)	
Vaginal pH				
Mean±SD	6.01±0.4	6.17±0.24	5.62±0.46	0.001a
Median (Range)	6 (5-6.5)	6 (6-6.5)	6 (5-6)	

No. : Number of cases; SD: standard deviation; BMI: Body mass index; a: p-value by unpaired t-test, b: p-value by Fisher exact test,*p-value by Mann Whitney test

Table 2 shows the parameters of the oocytes as well as the number of transferred embryos. The total number of oocytes varied between 2 and 25. The mean number of oocytes was not statistically different between non-pregnant and pregnant women (P =0.548). Furthermore, there was no difference in mean MII oocyte number between the non-pregnant and pregnant groups (P =0.582).

There was no significant difference in the mean number of oocyte fertilization between the two groups (P =0.769). Furthermore, no significant difference in the mean number of transported embryos was seen between the non-pregnant and pregnant females (P =0.740).

Table (2): oocyte and embryo characteristics of infertile women involved in this study.

Parameter	Total Number of cases(46)	Not pregnant (33)	Pregnant (13)	P-value
Total no. of oocyte				
Median (Range)	10 (2-25)	10 (2-25)	12 (3-21)	0.548
Mean±SD	11.28±6.09	10.94±6.26	12.15±5.77	
Total no. of MII oocyte				
Median (Range)	7 (1-19)	7 (1-19)	7 (2-14)	0.582*
Mean±SD	7.78±4.85	7.73±5.36	7.92±3.4	
No. of a fertilized oocyte				
Median (Range)	5 (1-14)	4 (1-14)	5 (2-10)	0.769*
Mean±SD	5.89±3.58	6.03±4.06	5.54±1.98	
No. of embryos transferred				
Median (Range)	3 (1-4)	3 (1-4)	3 (1-4)	0.740*
Mean±SD	2.74±1.2	2.73±1.31	2.77±0.93	

* P value by Mann Whitney test, others by unpaired t-test

Table 3 shows the polymicrobial community found by RT-PCR in the enrolling female lower reproductive tract. Gardnerella vaginalis was found in 38 of the cases (82.6 percent), Ureaplasma parvum in 19 (41.3 percent), Mycoplasma genitalium in 8 (17.4 percent), Ureaplasma urealyticum in 2, 4.3 percent), Neisseria gonorrhea in 1 (2.2 percent), Trichomonas vaginalis in 1 (2.2 percent), and 3 cases (6.5 percent) showed no microorganism. The microorganisms detected in the follicular fluid by RT-PCR are shown in Table (4).

Gardnerella vaginalis was found in 33 of the cases (71.7 percent), Ureaplasma parvum in 9 (19.6 percent), Mycoplasma genitalium in 5 (10.9 percent), Herpes simplex virus type-1 in 3 (6.5 percent), Ureaplasma urealyticum in 2 (4.3 percent), Mycoplasma hominis in 1 (2.2 percent), and 6 cases showed no microorganism (13 percent).

Table (3): Frequency and percentage of the vaginal microbiome.

Microorganism	No.	Percentage
Gardnerella vaginalis	38	82.6
Ureaplasma parvium	19	41.3
Mycoplasma genitalium	8	17.4
Ureaplasma urealyticum	2	4.3
Neisseria gonorrhea	1	2.2
Trichomonas vaginalis	1	2.2
No microorganism	3	6.5

Table (4): Frequency and percentage of follicular fluid microorganisms

Microorganism	No.	Percentage
Gardnerella vaginalis	33	71.7
Ureaplasma parvium	9	19.6
Mycoplasma genitalium	5	10.9
Herpes simplex virus1	3	6.5
Ureaplasma urealyticum	2	4.3
Mycoplasma hominis	1	2.2
No microorganism	6	13.0

Table 5 illustrates the frequency distribution and proportion of microorganisms detected by RT-PCR using DNA extraction in collected samples based on pregnancy outcome. Microorganisms were found in 43 (93 percent) of the vaginal samples, whereas none were found in 3 (7 percent). The most prevalent species was G. vaginalis, which had 38 cases, followed by U. parvum, which had 19 instances. Microorganisms were found in 40 (86.9%) of the follicular fluid samples, whereas none were found in 6 (13.1%) of the instances. G. vaginalis was the most common species, with 33 cases. U. parvum was next, with 9 cases.

Table (5): Comparison of frequency and percentage of microbiome obtained by RT-PCR through DNA extraction according to the pregnancy outcome of the cases.

Microorganisms identified in collected samples	No pregnancy (33) No.(%)	Pregnant (13) No. (%)	P value
Gardnerella vaginalis in vaginal sample	25 (75.8%)	13 (100%)	0.084
Gardnerella vaginalis in follicular fluid	23 (69.7%)	10 (76.9%)	0.729
Ureaplasma parvum in vaginal sample	14 (42.4%)	5 (38.5%)	1.000
Ureaplasma parvum in follicular fluid	6 (18.2%)	3 (23.1%)	0.698
Mycoplasma genitalium in vaginal sample	8 (24.2%)	0 (0.0%)	0.084
Mycoplasma genitalium in follicular fluid	5 (15.2%)	0 (0%)	0.301
Ureaplasma urealyticum in vaginal sample	2 (6.1%)	0 (0.0%)	1.000
Ureaplasma urealyticum in follicular fluid	2 (6.1%)	0 (0%)	1.000
Neisseria gonorrhea in vaginal sample	1 (3.0%)	0 (0.0%)	1.000
Neisseria gonorrhea in follicular fluid	0 (0%)	0 (0%)	
Trichomonas vaginalis in vaginal sample	1 (3.0%)	0 (0.0%)	1.000
Trichomonas vaginalis in follicular fluid	0 (0%)	0 (0%)	
Herpes simplex virus1 in vaginal sample	0 (0%)	0 (0%)	
Herpes simplex virus1 in follicular fluid	2 (6.1%)	1 (7.7%)	1.000
Mycoplasma hominis in vaginal sample	0 (0%)	0 (0%)	
Mycoplasma hominis in follicular fluid	1 (3%)	0 (0%)	1.000
No bacteria in a vaginal sample	3 (9.09%)	0 (0.0%)	0.548
No bacteria in follicular fluid	4 (12.12%)	2 (15.38%)	1.000

4. Discussion

The vaginal microbial makeup has a substantial impact on women's health. The use of metagenomics to explore the vaginal and follicular microbiota of asymptomatic infertile Iraqi women starting an ICSI trial for the first time during the current study.

New developments in molecular microbiology have made it possible to study the microbial profile of the genital tract in more depth. Even though the vaginal microbiota and bacterial vaginosis (BV) have been studied for a long time to prevent preterm delivery, the genital tract microbiome has gotten a lot of attention in recent years when it comes to early implantation, early pregnancy, and the number of live births.

Previously, it was considered that the endometrium was a sterile chamber, but current research suggests the opposite—that bacteria are prevalent in the endometrium **(Skafte-Holm et al., 2021 [11]).** This has led to the theory that vaginal bacteria can enter the endometrium and disrupt reproduction **(Haahr et al., 2020 [12]).**

Infertility caused by polymicrobial vaginal infection is a major public health concern all over the world. It has been associated with Chlamydia trachomatis, Mycoplasma species, and Neisseria gonorrhea, particularly due to endometrial and tubal dysfunction **(Li M. et al., 2017 [13]).** A large body of evidence implies that asymptomatic BV is spread sexually. Based on this, BV treatment should be investigated to avoid continuous sexual transmission to other partners **(Muzny and Schwebke, 2021 [14].**

BV was widespread among infertile patients in the current study and did not affect ICSI outcomes or clinical pregnancy rate, similarly to previous studies. Although the exact reason for infertility in BV patients is unknown, numerous explanations have been hypothesized. One possibility is that there is a link between BV microbiota and inflammation, which could lead to

less fertility. BV-related bacteria have been demonstrated to activate the immune system by maturing dendritic cells and increased levels of proinflammatory cytokines, culminating in mucosal inflammation of the reproductive tract **(Ravel et al., 2021 [1])**. The influence of sialidase and mucinase enzymes on cervical mucus integrity is another BV-related strategy that may lead to infertility **(Ravel et al., 2021 [1]).** Biofilm generation and antibiotic resistance of BV-associated bacteria like G. vaginalis may be determinants of persistence and recurrence **(Verwijs et al., 2020 [15]).**

During transvaginal oocyte collection, several microorganisms that are contaminants or colonizers were found in the follicular fluid. The research on the influence of bacterial content in follicular fluid on the results of assisted reproductive procedures is inconsistent. These different results could be due to several things, such as how the research was done, what the expected results would be, how many people were sampled, and what diagnostic method was used. In a recent study published in 2021, Usman et al. discovered that separating organisms from follicular fluid had no negative effect on conception and clinical pregnancy rates following ICSI cycles. **(Usman et al., 2021 [16]).**

6- Conclusion

According to the findings, the vaginal micro-ecology profile enabled the detection of unknown vaginal dysbiosis shifts throughout the ICSI-ET procedure. According to the abnormal vaginal micro-ecology profile, most women do not have a clinically visible infection. A thorough study of the vaginal microbial profile in ART may lead to tailored therapy approaches, such as vaginal antibiotic administration and pre-or probiotics, to particularly modulate microbiota to a more normal profile. Even though there are germs in follicular fluid, isolating microorganisms from follicular fluid

did not hurt the chances of fertilization or getting pregnant during ICSI cycles.

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Author Contribution

Saja F. Faisal performed the study, and Wasan A. Abdul Hameed, Sarab H. Abdulhussain, and Estabraq Alwasiti supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

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