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Original Article

The Influence of Vaginal Dysbiosis on Intracytoplasmic Sperm Injection Outcome

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Abstract

In women of reproductive age, vaginal infection is a gynaecological condition with various health consequences. Bacterial vaginosis, vulvovaginal candidiasis, and aerobic vaginitis are the most prevalent types of infection. Although reproductive tract infections are known to impact human fertility, no consensus guidelines on microbial control in infertile couples undergoing *in vitro* fertilization therapy are currently available. This study aimed to determine the effect of asymptomatic vaginal infections on the outcome of intracytoplasmic sperm injection in infertile Iraqi couples. Forty-six asymptomatic infertile Iraqi women were evaluated for genital tract infections by taking a vaginal sample on ovum pick-up for microbiological culture during their intracytoplasmic sperm injection treatment cycle. Based on the acquired results, a multi-microbial community colonized the participant's female lower reproductive tract, and only 13 women achieved pregnancy compared to 33 who did not. *Candida albicans* was found in 43.5% of the cases, 39.1% *Streptococcus agalactiae*, 19.6% *Enterobacter species*, 13.0% *Lactobacillus*, 8.7% *Escherichia coli*, 8.7% *Staphylococcus aureus*, 4.3% *Klebsiella*, and 2.2% *Neisseria gonorrh*oeae. However, no statistically significant effect was observed on the pregnancy rate except for *Enterobacter* spp. and *Lactobacilli*. In conclusion, the majority of patients had a genital tract infection; *Enterobacter* spp. had a substantial negative influence on the pregnancy rate, and *lactobacilli* were highly related to positive outcomes in participating females.

Keywords: Infertility, Vaginaldysbiosis, Lactobacilli, ICSI

1. Introduction

Although the microbiome may significantly impact host biology, little is understood about the microbial populations in male and female reproductive systems (1). The kind of vaginal microbiota frequently influences pregnancy outcomes and women's health. This microbiota comprises many different types of bacteria in different amounts and ratios that can prevent or induce various vaginal infections (2).

Lactobacilli predominate in the vaginal microbiome of most healthy women. While a change in microbial populations, including the increasing replacement of specific *Lactobacillus* species by virulence factors or opportunistic microbes, typically characterizes vaginal infections. Bacterial vaginosis (BV), aerobic vaginitis (AV), and vulvovaginal candidiasis (VC) are some of the vaginal diseases that can be caused by this microbial shift (2).

Optimal vaginal microbiota occurs in symbiotic relationships and is thought to guard against pathogen colonization and infection by forming lactic acid and antimicrobial metabolites and through low-level immune system activation. The ascendance of bacterial pathogens and other anaerobic bacteria has increased the risk of sexually transmitted infections (STIs) and upper reproductive tract infections when the

preponderance of *lactobacilli* is disrupted (3). Changes in the microbiota composition of the human vagina can occur during several life phases, including birth, puberty, pregnancy, and menopause (4). Indeed, hormonal shifts, uncontrolled antibiotic use, menstruation, and vaginal douching are all prevalent variables influencing the temporal alterations in human vaginal microbiota (5).

Among women of reproductive age, vaginal infection is the most common gynaecological condition. It affects millions yearly and is the main reason for gynaecological medical treatment (2). Aerobic vaginitis (AV) is a condition in which the microbiota changes from *Lactobacillus* to *enterobacteria*, *staphylococci*, *streptococci*, and *enterococci*, resulting in an inflammatory condition (6).

Assisted reproductive technologies refer to any technique that includes the *in vitro* manipulation of human oocytes, sperm cells, or embryos to generate a pregnancy (7, 8). Intracytoplasmic sperm injection (ICSI), previously favoured over other methods to treat male factor infertility, is now used to treat advanced maternal age and idiopathic infertility. ICSI now accounts for approximately 70–80% of total assisted reproductive cycles, making it the most widely used therapy (9). In IVF, it has been found that the vaginal microbial composition on the day of embryo transfer has a significant impact on the success rate (10), and the most favourable scenario for successful IVF is a lactobacilli-dominated microbiome (11).

The aim of this study was to evaluate the effect of asymptomatic vaginal infections on the outcome of intracytoplasmic sperm injection in infertile Iraqi couples.

2. Materials and Methods

This is cross-sectional research conducted at Al Nahrain University's Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies between November 2020 and November 2021. Each patient supplied written informed permission before participating in the experiment. The study involved forty-six infertile Iraqi women between the ages of 21 and 47 undergoing controlled ovarian hyperstimulation for ICSI treatment cycles. They were asymptomatic for vaginal infections and had fresh embryo transfers. Women treated with broad-spectrum antibiotics or vaginal therapies before having controlled ovarian hyperstimulation procedures or with abnormal vaginal secretions were excluded from the study.

2.1. Sample Collection

On the day of oocyte retrieval, vaginal samples were collected using cotton wool swabs and placed in transferring media for microbiological culture. The pH of vaginal discharge was measured using colour-fixed indicator strips (URS-3) from Henson Medical (China).

2.2. Specimens Culturing

Agar plates were used to plate the specimens. In a sterile setting, a variety of culture agar plates (horse blood agar, chocolate agar, MacConkey agar, Sabouraud Dextrose agar) from (Oxoid, UK) were seeded. Plate media were incubated either aerobically at 5% CO_2 or anaerobically for 24 hours at 37°C (12). A thin layer of the specimen was put on a clean glass slide and allowed to dry on air before being heat-fixed and Gram-stained for bacterial identification (13) based on colour and morphology under the microscope.

2.3. Controlled Ovarian Hyperstimulation

Multiple follicle growth was accomplished using 150–300 IU of the follicular stimulating hormone (Gonal FR, Merk, Switzerland), depending on ovarian response as evaluated by hormone blood levels and ultrasound examination. Once the larger follicle reached 14 mm in diameter, a Gonadotropin-Releasing Hormone (GnRH) antagonist (Cetrorelix acetate for injection 0.25 mg: Cetrotide®, Merk, Switzerland) was administered daily. When a minimum of three follicles larger than 16 mm in diameter were observed, ovulation was induced by 10,000 IU of human chorionic gonadotrophins (hCG) intramuscular injections (Pregnyl®, Organon, Holland), and34–36 hours later, oocyte collection was scheduled. Intracytoplasmic sperm injection was carried out to fertilize the oocytes, and embryo transfer (ET) took

place 3–5 days later. Serum hCG levels were measured 14 days after the ET. A transvaginal ultrasound was done at weeks 6-7 of pregnancy to see if there was a gestational sac.

2.4. Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 23 and Microsoft Office Excel 2019 were used. The qualitative parameters were reported as frequencies and percentages, and the Fisher exact test was used to compare the ICSI outcome. Whereas (vaginal PH) was first tested for normality distribution using the Shapiro-Wilk test and expressed as mean and standard deviation, the unpaired t-test was used to compare pregnant and non-pregnant women. A *P*-value of less than 0.05 was determined to be significant.

3. Results

The frequency and percentage of the multi-microbial community colonizing the participant's female lower reproductive tract as found by utilizing the cultivable technique is shown in table 1.

Table 2 demonstrates the frequency and percentage of vaginal microbiomes found through cultivable techniques based on ICSI results. Patients were grouped according to ICSI outcome into those pregnant or not.

There was no statistically significant difference in the frequency distribution of Candida albicans, Streptococcus agalactiae, Escherichia coli, Staphylococcus aureus, Klebsiella, and Neisseria gonorrhoea between non-pregnant and pregnant groups ($P \geq 0.05$). However, there was a significant difference in the frequency distribution of Enterobacter spp. ($P=0.044$) between non-pregnant 9 (27.3%) and pregnant groups 0 (0.0%). Furthermore, Lactobacillus species (spp.) revealed a statistically significant difference in frequency distribution with $P=0.045$ between non-pregnant 2 (6.1%) and pregnant 4 (30.8%) groups.

Table 3 shows the vaginal PH values on the day of oocyte retrieval; there was a statistically significant difference between the two groups ($P=0.001$), in which the non-pregnant vaginal PH was (6.17±0.24) compared to the pregnant group (5.62±0.46).

Table 1. The prevalence of vaginal microbiome obtained by microbiological culture

Bacteria	Frequency	Percentage
Candida albicans	20	43.5%
Streptococcus agalactiae	18	39.1%
Enterobacter spp.	9	19.6%
Lactobacillus	6	13.0%
Escherichia coli	4	8.7%
Staphylococcus aureus	4	8.7%
Klebsiella	2	4.3%
Neisseria gonorrhoea	1	2.2%

The total number of cases is 46; some cases have more than one species of bacteria

Table 2. The prevalence of vaginal microbiome obtained by microbiological culture according to ICSI outcome

Bacteria	not pregnant (33) No. (%)	Pregnant (13) No. (%)	*P*-value
Candida albicans	14 (42.4%)	6 (46.2%)	1.000
Streptococcus agalactiae	13 (39.4%)	5 (38.5%)	1.000
Enterobacter spp.	9 (27.3%)	0 (0.0%)	0.044
Lactobacillus spp.	2 (6.1%)	4 (30.8%)	0.045
Escherichia coli	3 (9.1%)	1 (7.7%)	1.000
Staphylococcus aureus	4 (12.1%)	0 (0.0%)	0.313
Klebsiella	1 (3.0%)	1 (7.7%)	0.490
Neisseria gonorrhoea	1 (3.0%)	0 (0.0%)	1.000

No. (number) of cases 46, some cases have more than one species of bacteria, p-value Fisher exact test

Table 3. Vaginal PH among pregnant and non-pregnant women

Parameter		Total number (46)	not pregnant (33)	Pregnant (13)	*P*-value
Vaginal pH	(Range)	(5-6.5)	(6-6.5)	(5-6)	0.001
	Mean±SD	6.01±0.4	6.17±0.24	5.62±0.46	

P-value by unpaired t-test

4. Discussion

Although assisted reproductive technologies have enabled certain obstacles to be solved, conception rates remain low. Various reasons, including genital tract infections, can cause infertility. However, there are currently no consensus recommendations on microbiological examination of infertile couples preceded IVF (9).

The current study investigated the impact of asymptomatic genital tract infections on female fertility, and the key findings were as follows:

(i) The majority of patients had a genital tract infection; (ii) Enterobacter spp. had a significant negative impact on ICSI outcomes; (iii) the presence of lactobacilli in females was strongly associated with a favourable ICSI outcome, and vaginal PH was more acidic in pregnant women.

Despite testing positive for microbiological infections, all of the infertile women in the study had no symptoms of vaginal tract infections. The microbial species found in our study are primarily vaginal colonizers. In this study, Candida albicans was determined to be the most common pathogen detected (43.5%) and was shown not to impact fertility.

Endometrial dysbiosis and ICSI outcome failure have long been researched utilizing microbiological cultures of embryo transfer catheter tips. Since the mid-1990s, the identification of Enterobacteriaceae, streptococci, staphylococci, enterococci, and/or gram-negative bacteria has been linked to reduced implantation and pregnancy rates as well as increased miscarriage rates, whereas the identification of lactobacilli or specimens with negative cultures for the previously mentioned pathogens has been linked to better reproductive outcomes (14). Enterobacter spp. can have a deleterious impact on reproduction by directly injuring the genital tract mucosa, eliciting inflammatory responses in the host, or indirectly altering the functional property of the genital tract by creating toxic metabolites. Furthermore, these bacteria have been documented to change several sperm characteristics such as motility, viability, morphology, acrosomal reaction, and so on, all of which are determinants of infertility (9). Many possible mechanisms for lactobacilli's probiotic effects have been hypothesized, including competition for colonization, regulation of host immunological response, cross-feeding of commensal flora, synthesis, and excretion of lactase, bile salt hydrolase, antimicrobial substances, and organic acids. Lactobacillus surface-active molecules (SAMs) are thought to be responsible for the probiotic properties of lactobacilli. It affects the body's immune system by adhering to surface epithelial cells and pattern recognition receptors (PRR) on the mucosal layer. Lactobacilli SAMs with anti-biofilm, antioxidant activity, antiviral, pathogenic-inhibition, and immunomodulation activities against vaginal pathogens are postulated to be directly implicated in the interaction between the host and vaginal microbiota (15).

The naturally neutral pH is 7, while the normal vaginal pH ranges from 3.8 to 5.0, indicating a somewhat acidic environment. It plays an essential part in vaginal health, but it is worth noting that maintaining an excellent vaginal pH is determined by the metabolism of Lactobacillus species and other commensal flora, estrogen, glycogen, and preexisting pathogens (16). The overall health conditions, such as

age, vaginal hydration state, daily food, and safe intercourse, can all impact vaginal pH (16). It is an important helpful sign of vaginitis. There is a higher chance of vaginitis if there is an abnormal pH reading, and measuring vaginal pH can help with early detection (17). Even in the absence of active vaginal infection, high vaginal pH can lead to unfavourable pregnancy results through an associated systemic inflammatory response (18).

More research is needed in this area to improve the management of genital tract disorders such as infection in infertile couples; it may aid in increasing conception rates, reducing the total number of treatment cycles, and may improve fertility techniques, all of which may benefit the couple's well-being and healthcare expenses.

Most patients had a genital tract infection even if they were asymptomatic; Enterobacter spp. had a substantial negative influence on the pregnancy rate, while lactobacilli were highly related to positive reproductive outcomes in participating females, and acidic vaginal pH was associated with a higher pregnancy rate.

Authors' Contribution

Study concept and design: S. F. F.
Acquisition of data: W. A. A. H.
Analysis and interpretation of data: E. A.
Drafting of the manuscript: W. A. A. H.
Critical revision of the manuscript for important intellectual content: S. F. F.
Statistical analysis: W. A. A. H.
Administrative, technical, and material support: E. A.

Ethics

The committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, approved the study on 01.11.2020 and approval number: (2/3/783).

Conflict of Interest

The authors declare that they have no conflict of interest.

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