The Prevalence of Asymptomatic Sexually Transmitted Infections in the Female Reproductive System and its Impact on Infertile Iraqi Women Starting an ICSI Trial:

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Abstract:

Infertility is a disorder that has generally been characterized as the inability to obtain a fruitful pregnancy following 12 months of unprotected sex. The resident microbiome within the genital tract can help to create a good environment for conception, as well as fight off harmful factors like sexually transmitted diseases. Infertile couples can be a useful study model for evaluating the microbial markers impacting reproductive health. Couples are examined for the presence of sexually transmitted diseases such as hepatitis B and C, as well as the human immunodeficiency virus before in-vitro fertilization cycles, but routine microbiological testing is not performed. The purpose of this study is to evaluate if the presence of sexually transmitted microbiomes in follicular fluid and vaginal samples of asymptomatic Iraqi women undergoing intracytoplasmic sperm injection, affects fertilization and pregnancy rate. The result revealed that follicular fluid isn't germ-free, but the isolation of microorganisms from follicular fluid did not adversely affect intracytoplasmic sperm injection outcomes.

Keywords: Infertility**,** Sexually transmitted infections, ICSI, Colonization of follicular fluid.

1. Introduction

Infertility is a complicated condition that causes substantial medical, emotional and financial issues. It is described as a couple's failure to conceive after 12–24 months of regular unprotected sexual intimacy if the mother is under the age of 35, and after six months of unprotected regular sexual relations if the mother is over the age of 35[1]. It involves 48.5 million married couples worldwide [2]. One-third of infertility cases are caused by female causes, another third by malefactors, and the other third are caused by both spouses and unexplained infertility [3].

Female infertility is caused predominantly by ovulatory disorders; decreased ovarian reserve; reproductive system anatomical, endocrine, genetic, functional, or immunological anomalies; chronic medical problems; and sexual circumstances incompatible with coitus[4].

Infections including Neisseria gonorrhea and Chlamydia trachomatis are major causes of pelvic inflammatory disease, resulting in pelvic adhesions and, ultimately, tubal infertility [5]. Infertile couples who use assisted reproductive technologies can be a useful study model for evaluating the microbial markers impacting reproductive health. As part of this, it's important to think about how a resident microbiome can help to create a good environment for conception, as well as how it can fight off harmful factors like sexually transmitted diseases [6].

Couples are examined for the presence of sexually transmitted diseases such as hepatitis B and C, as well as the human immunodeficiency virus (HIV) before intracytoplasmic sperm injection (ICSI) cycles, but routine microbiological testing is not performed. One of the most challenging circumstances is when embryo contamination happens after a significant investment of money and time. When germs from contaminated embryo culture material enter the female reproductive system, they can cause unfavorable pregnancy outcomes. Furthermore, bacteria from follicular fluid may cause embryo contamination (7).

As far as we know, no similar research on this issue has been conducted in Iraq, and because of the large number of infertile individuals who undergo assisted reproduction techniques, this type of study is vital and will provide a crucial piece of information that can help physicians improve the method. It will also lay the groundwork for future research into regular follicular fluid screening and links between follicular fluid bacteria and unexplained infertility.

2. Materials and Methods

This is a cross-sectional study carried out at the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al Nahrain University in Baghdad, Iraq between November 2020 and November 2021. It was authorized by the local medical ethical council, and each patient provided written informed consent before participating in the trial.

Forty-six women between the ages of 21 and 47, who didn't have a vaginal infection, were stimulated with an antagonist protocol, and had fresh embryo transfers, were all included in the study. Women who had been given broad-spectrum antibiotics or local vaginal treatments before the start of controlled ovarian hyperstimulation protocols, or who had abnormal vaginal secretions, were not included.

Basic steps in the ICSI treatment cycle

Using follicle-stimulating hormone at a dosage of 150–300 IU dependent on ovarian response as measured by hormone blood levels and ultrasound inspection, multiple follicle growth was achieved. A Gonadotropin-Releasing Hormone (GnRH) antagonist was given daily once the dominant follicle reached 14 mm in diameter. Ovulation was triggered by injecting human chorionic gonadotropin (hCG) when at least three follicles larger than 16 mm were present. The oocyte collection was planned for 34–36 hours following the hCG injection. ICSI was used to fertilize oocytes, and embryo transfer (ET) was performed 3–5 days later. Fourteen days after the ET, serum hCG levels were checked. A transvaginal ultrasound was performed at weeks 6-7 of pregnancy to ensure that a gestational sac was present.

Sample collection:

High vaginal swabs were obtained at the time of ova collection for DNA extraction, and after the egg was extracted, follicular fluid was collected for DNA extraction to determine the presence of sexually transmitted microbiomes.

Real-time PCR was used to look for genital tract infections with exacting growth requirements or that are non-cultivable, such as Chlamydia trachomatis, Neisseria gonorrhea, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Mycoplasma genitalium, Trichomonas vaginalis, Gardnerella vaginalis, and herpes simplex virus (HSV) types (1 & 2) using a commercial kit.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 23 and Microsoft Office Excel 2019 were used to collect, summarize, analyze, and present data. Qualitative (categorical) variables were expressed as numbers and percentages, and a comparison between pregnant and non-pregnant women was done using Fisher's exact test. The level of significance was considered to be a P-value of less than 0.05.

3. Results

Table 1 shows the relationship between vaginal microorganisms and pregnancy outcomes. The total number of cases was 46. The positive pregnancy outcomes were 13 (28.3%), while 33 (71.7%) were not pregnant.

Most cases have more than one species of bacteria. Microorganisms were detected in 43 (93%) of the vaginal samples, while 3 (7%) of the cases showed no microorganisms. The identified microorganisms were Gardnerella vaginalis, Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma genitalium, Neisseria gonorrhea, and Trichomonas vaginalis.

As shown in table (1), Gardnerella vaginalis was the most common species, with 38 cases, followed by Ureaplasma parvum with 19 cases. Among groups that were not pregnant or pregnant, there was no statistically significant difference in the frequency and percentage of microorganisms found.

Table (1): The relationship between vaginal microorganisms and pregnancy outcomes

vaginal microorganisms	Not pregnant No. (%) (33)71.7%	Pregnant No. (%) (13)28.3%	P value
Gardnerella vaginalis	25 (75.8%)	13 (100%)	0.084
Ureaplasma parvum	14 (42.4%)	5 (38.5%)	1.000
Mycoplasma genitalium	8 (24.2%)	0 (0.0%)	0.084
Ureaplasma urealyticum	2 (6.1%)	0 (0.0%)	1.000
Neisseria gonorrhea	1 (3.0%)	0 (0.0%)	1.000
Trichomonas vaginalis	1 (3.0%)	0 (0.0%)	1.000
No bacteria	3 (9.09%)	0 (0.0%)	0.548

No. of cases 46, some cases have more than one species of bacteria

Table 2 shows the relationship between follicular fluid microbiomes and ICSI outcomes (pregnant or not). Most cases have more than one species of bacteria. Microorganisms were detected in 40 (86.9%) of the follicular fluid samples, while 6 (13.1%) of the cases showed no microorganisms. The identified microorganisms were Gardnerella vaginalis, Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma genitalium, Mycoplasma hominis, and Herpes simplex virus type-1. Gardnerella vaginalis was the most common species, with 33 cases, followed by U. parvum with 9 cases. Also, there was no statistically significant difference in the frequency distribution and percentage of recognized microorganisms among non-pregnant and pregnant groups.

Table (2): The relationship between follicular fluid microorganisms and pregnancy outcomes

Microorganisms present in Follicular fluid	**Not pregnant (33) 71.7% No. (%)**	**Pregnant (13) 28.3% No. (%)**	**P-value**
Gardnerella vaginalis	23 (69.7%)	10 (76.9%)	0.729
Ureaplasma parvum	6 (18.2%)	3 (23.1%)	0.698
Mycoplasma genitalium	5 (15.2%)	0 (0%)	0.301
Herpes simplex virus1	2 (6.1%)	1 (7.7%)	1.000
Ureaplasma urealyticum	2 (6.1%)	0 (0%)	1.000
Mycoplasma hominis	1 (3%)	0 (0%)	1.000
No bacteria	4 (12.12%)	2 (15.38%)	1.000

No. of cases 46, some cases have more than one species of bacteria

Table 3 shows the frequency and percentage of colonized microorganisms in a follicular fluid according to RT-PCR results. Microbiomes which were recognized only in follicular fluid with no vaginal existence (colonized) were Gardnerella vaginalis, present in one case only, Ureaplasma parvum in 2 cases, Mycoplasma genitalium in 2 cases, Herpes simplex virus type-1 was found in 3 cases only in follicular fluid with no vaginal existence, Ureaplasma urealyticum in one case, and Mycoplasma hominis in one case.

Table (3): Frequency and percentage of colonized microorganisms in follicular fluid

Microorganism present in Follicular fluid	No. of cases	Vaginal Microorganism	
		Present	**Absent**
Gardnerella vaginalis	33	32 (97.0%)	1 (3.0%)
Ureaplasma parvum	9	7 (77.8%)	2 (22.2%)
Mycoplasma genitalium	5	3 (60.0%)	2 (40.0%)
Herpes simplex virus1	3	0 (0.0%)	3 (100%)
Ureaplasma urealyticum	2	1 (50.0%)	1 (50.0%)
Mycoplasma hominis	1	0 (0.0%)	1 (100%)
No microorganism	6	2 (33.3%)	4 (66.7%)

No. of cases 46, some cases have more than one species of bacteria

4. Discussion

Women's health is significantly influenced by their vaginal microbial composition. The current study is the first to employ metagenomics to investigate the vaginal microbiome of infertile Iraqi women who are asymptomatic.

Vaginal samples may be acquired non-invasively, and the microbial load in the vagina is several times greater than that in the uterus. Any microbes found in the uterus must be regarded as an overspill from the vaginal microbiome. Most women had a vaginal microbiome dominated by Lactobacilli, which are linked to a balanced immunological tolerant vaginal milieu. Lactobacillus crispatus, to be specific, does not cause vaginal mucosal irritation and is also linked to pathogen defense[8].

Infertility induced by polymicrobial vaginal infection is a serious public health issue across the world. Chlamydia trachomatis, Mycoplasma spp., and Neisseria gonorrhea have all been linked to infertility, mostly due to endometrial and tubal inflammation [9].

An increase in the presence of specific bacteria (Atopobium vaginae, Ureaplasma parvum, Ureaplasma urealyticum, and Gardnerella), which is normally seen in asymptomatic bacterial vaginosis, as well as an increase in Candida and a decrease in vaginal and cervical Lactobacillus, is frequently observed in women with fertility problems [10].

The current study showed that Gardnerella vaginalis was the most common type of bacteria in non-pregnant women (75.8 percent) and pregnant women (100 percent), with no statistically significant difference (Table 1).

The pathogenesis of asymptomatic bacterial vaginosis (BV) is unknown, even though it is common. BV-related bacteria have been demonstrated to activate the immune system by maturing dendritic cells and increased levels of proinflammatory cytokines, resulting in mucosal inflammation of the reproductive tract. Cervical interleukin (IL)-1b, IL-6, and IL-8 cytokines are higher in infertile women with BV[11].

A significant body of data suggests that asymptomatic BV is transmitted sexually. Based on this, BV therapy should be explored to prevent the ongoing sexual spread of the BV pathogens to other sexual partners [12]. In the current study, BV did not affect ICSI results or clinical pregnancy rate, which was consistent with earlier research.

Several bacteria have been found in follicular fluids collected during transvaginal oocyte extraction and are classified as contaminants or colonizers. Contaminants are microorganisms found in both follicular fluid and vaginal samples taken from the same woman. Colonizers are germs that appear in follicular fluid but not in vaginal samples [13]. According to Tomaiuolo et al., ovarian blood supply may facilitate the hematogenous transit of germs from other anatomical regions [14]. A study by Pelzer et al. in 2013 suggested that microbiomes from other parts of the body could move to the genital tract through blood flow. This could include bacteria from the lung or the Herpes simplex virus type-1 from the oral cavity[15].

The existing evidence on the effect of follicular fluid bacterial content on assisted reproductive techniques results is contradictory. These contradictory results might be attributed to a variety of factors, including research design, targeted demographic, predicted outcomes, sampling size, and the diagnostic procedure utilized.

In recent research published in 2021, Usman et al. determined that isolating microorganisms from follicular fluid had no deleterious effect on fertilization and clinical pregnancy rates following ICSI treatment cycles [13].

In the current research following earlier studies, there was no significant difference between both the pregnant and non-pregnant groups and follicular fluid microbial colonization had no negative effect on ICSI outcome (Table 2).

5. Conclusions

Follicular fluid isn't germ-free, but the isolation of microorganisms from follicular fluid did not adversely affect fertilization and pregnancy rates following ICSI treatment cycles.

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