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RESEARCH ARTICLE

The Role of Asymptomatic Bacterial Vaginosis in Women Undergoing Intracytoplasmic Sperm Injection in Predicting Successful Pregnancy

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Abstract

Background: Bacterial vaginosis (BV)is a polymicrobial clinical syndrome resulting from replacement of the normal hydrogen peroxide producing Lactobacillus sp. BV is the most common lower genital tract disorder among women of reproductive age (pregnant and non-pregnant) and the most prevalent cause of vaginal discharge and malodor.

Objectives: To study the role of asymptomatic bacterial vaginosis on outcome in patients undergoing ICSI.

Patients and Methods: This prospective study was undertaken in the High Institute of Infertility Diagnosis and Assisted Reproductive Techniques / Al-Nahrain University. During the period from September 2017 – April 2018. A total of 100 infertile women .All of them were underwent controlled ovarian hyper stimulation for intra cytoplasmic sperm injection cycle. Antagonist oocyte maturation used as ovulation induction protocol in all the cases. Assessment of oocyte maturation and embryo quality is done to the all the cases, bacterial vaginosis smear was collected on the day of oocyte retrieval. B.V. smear diagnosis by Amsel's Criteria and Nugent's Scoring.

Results: TThere was no significant difference in mean age of pregnant and non-pregnant ladies, 29.40 ±6.47 years versus 30.78±6.84 years, respectively (P= 0.333).In addition the frequency of women under 35 years age was comparable in the two groups with no statistical significant difference, 34 (79.1%) versus 33 (73.3%), respectively (P = 0.528). Wide variation and non-normal distribution was the role followed by the duration of infertility concerning sub-fertile women participating in this study. However, no significant difference was observed in the duration of infertility between pregnant and non-pregnant ladies, 6.00 (5.00) years versus 6.00 (5.00) years (P = 0.289). Comparison of basal hormonal levels between pregnant and non-pregnant groups revealed no significant difference despite the presence of some minor differences in mean hormonal levels between the two groups (P> 0.05). Comparison of oocytes characteristics between pregnant and non-pregnant ladies was conducted and revealed no significant difference in mean total number retrieved oocyte (TNO), ruptured oocytes (RO), abnormal oocyte (AO), germinal vesicle oocyte (GV), metaphase I oocyte and metaphase II oocytes (P > 0.05), table Mean total number retrieved oocyte (TNO), ruptured oocytes (RO), abnormal oocyte (AO), germinal vesicle oocyte (GV), metaphase I oocyte and metaphase II oocytes was 10.81 ±4.95 versus

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10.42±6.30, 0.44 ±0.73 versus 0.36 ±0.65, 1.09 ±1.56 versus 0.84 ±2.00, 0.91 ±1.00 versus 0.89 ±1.47, 1.51 ±1.49 versus 2.04 ±2.09 and 6.84 ±4.01 versus 6.22 ±3.91 in pregnant and non-pregnant groups, respectively. Mean fertilization rate was higher in pregnant ladies than that of non-pregnant ladies, 62.19 ±22.23 versus 57.59 ±24.95, respectively; however, the difference was no statistically significant (P = 0.365).Mean grade I embryo number was also significantly higher in pregnant women than that of non-pregnant women, 2.53 ±1.53 versus 1.89 ±1.27, respectively (P = 0.034), Mean grade II embryo number was 0.86 ±1.52 versus 0.89 ±1.28 in pregnant and non-pregnant ladies, respectively and the difference was not significant (P = 0.925), moreover the difference in mean grade III embryo number between pregnant and non-pregnant women was insignificant (P = 0.321), 0.30 ±1.01 versus 0.31 ±0.50, respectively. Rate of asymptomatic bacterial vaginosis as determined by gram stain, whiff test, wet mount and Amsel's criteria was 32.6 % in pregnant women compared to 73.3% in non-pregnant group, being highly significant higher in those women who failed to get pregnant (P<0.001).

Conclusion: Rate of asymptomatic bacterial vaginosis was 32.6 % in pregnant women compared to 73.3% in non- pregnant group, being highly significant higher in those women who failed to get pregnant.

Keywords: IVF, Bacterial vaginosis, oocyte & ICSI

Introduction:

Infertility can be described as the inability to become pregnant, maintain a pregnancy, or carry a pregnancy to live birth (1). Is a condition that affects approximately 1 out of every 7 couples(2). An infertility diagnosis is given to a couple that has been unsuccessful in efforts to conceive over the course of one full year. When the cause of infertility exists within the female partner, it is referred to as female infertility.

Bacterial Vagionosis (BV): is the most common lower genital tract disorder among women of reproductive age (pregnant and non-pregnant) and the most prevalent cause of vaginal discharge and malodor (3). It has been associated with a significant number of obstetric and gynecologic complications, such as preterm labour and delivery, preterm premature rupture of membranes, spontaneous abortion (4,5). 50% of all women with bacterial vaginosis are free of symptoms.

In vitro fertilization(IVF):has been proven to be highly effective therapy for treating infertility and childless couples with a variety of etiologic causes (female or male factor infertility, or combined). Intracytoplasmic sperm injection (ICSI) is more expensive than IVF and time consuming, there for efforts have been made to set the factors which predict a successful outcome in a given patient or couple.

Bacterial Vaginosis and Infertility:The main causes of pelvic inflammatory disease (PID), and hence tubal infertility, are Chlamydia trachomatis and Neisseria gonorrhoeae. However; bacterial vaginosis is(BV) is being increasingly implicated in upper genital tract infections in women. It can cause ascending infection in pregnant women, the chorioamnionitis, then predisposing to preterm delivery.

It also increases the rate of PID Following surgical termination of pregnancy. Non pregnant women with BV, but without gonorrhoea orchlamydia and without any symptoms or signs of upper genital tract inflammation. Symptoms of abdominal pain and inter menstrual bleeding are more common in women with BV than those with normal flora. BV is usually present in women with acute salpingitis, and BV-associated organisms are commonly isolated from the upper genital tract of patients with PID. They are usually found as co-infections with C. trachomatis and N. gonorrhoeae, but they have sometimes been isolated from the upper genital tract in the absence of these, with the suggestion

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that they increase the risk of upper genital tract infection in women with and without sexually transmitted infections. There is therefore mounting evidence that BV may cause PID in non-pregnant women in the absence of gonorrheae and chlamydia. Consequently, BV could be associated with tubal factor infertility (6).

Patients, Materials and Methods:

Patients

One hundred infertile couples underwent ICSI cycles. The study was approved by Local Medical Ethical Committee of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University ; Women> 41 years, Abnormal uterine cavity due to polypmyoma, or congenital anomalies, Uncontrolled systemic disease as diabetes mellitus or uncontrolled endocrinological disorder were excluded. All patients were subjected to full history taking, complete physical examination, measurement of BMI, basal hormone level (FSH, LH, LH/FSH ratio , prolactin, and estradiol) in cycle day 2 and estradiol at the day of hCG injection.

All patients were enrolled in antagonist protocol, on day 2 of menstrual period, a basic evaluation was conducted by ultrasound examination. Medication was then initiated with recombinant FSH (rFSH) (Gonal-F, EMD Serono), starting dose of (rhFSH) depends on women´s age, BMI, antral follicles count and previous response to ovulation induction . The follicles growth was monitored by serum E2 level and Trans – vaginal ultrasound. The GnRH antagonist, cetrorelix, was next administered daily by S.C. injection (0.25 mg/d) in the morning (8:00-12:00 AM) when the lead follicle reached 13mm till the day of human chorionic gonadotropin (hCG) administration. Sub sequent scan was done every 2-3days as required, till the day of hCG administration. Ovulation triggering was induced by the administration of recombinant hCG (rhCG 6500 IU, Ovitrelle®; Merck Serono) subcutaneously when two or more follicles had reached 18 mm(7) .

ICSI procedures and Bacterial vaginal smear collection

Trans-vaginal oocytes retrieval was performed 34-36 hours after hCG injection with ultrasound guidance. A diagnosis of asymptomatic bacterial vaginosis was made in this study according to:

1.Amsel criteria

When three of the following characteristics were detected:

q1-Thin, white, yellow, homogeneous discharge

2-Vaginal wet mount

A vaginal wet mount (or vaginal smear(8) or wet prep(9)) Vaginal discharge was collected on a cotton tipped swabs and placed into a test tube containing between 0.5 and 1 mL of normal saline.(8) .

One drop was placed onto a glass microscope slide and covered with a coverslip. The slide was examined on low (10X) power under low light within 1 hour of collection for detecting the clue cells. The 40X objective used to confirm the presence or absence of T vaginalis, G vaginalis, and yeasts.

3-Whiff test

A Whiff test several drops of 10 % a potassium hydroxide (KOH) solution was added to a sample of the vaginal discharge to test for any resultant strong fishy (amine) odor from the mix, which would indicate bacterial vaginosis.

4 -vaginal pH>>4.5

5-Gram Stain Procedure (Nugent's method)

The glass slide that contains the specimen was heat-fixed and had been flooded with crystal violet stain and remained in it for 1 min then rinsed gently under running tap water; next the slid was flooded in iodine solution and remained in it for 1 min.

Then rinsed gently under running tap water after that the slid was decolorized by letting the alcohol/acetone solution flow over the smear, while the slide was holed at an angle the de-colorize reapplying

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was stopped when the solution runs clear, then gently rinsed with running tap water. The slide was flooded in safrnin for 30 sec, then gently rinsed under running tap water, then kept in upright position, to be air-dried. The slide was scanned using a low power objective to locate any clusters of epithelial cells. When the flora had been noted in any area the oil immersion lens (x1000) was used for examining between 10 and 20 representative fields. '[Cell morphology and Gram reaction was observed by Nugent's method (1991)The BV score for Gram staining had been calculated . Per oil immersion field, the average number of lactobacilliary morphotypes had been quantified. That organisms were unusually filamentous, gram positive rods of varying length that often form chains, but occasionally, they might stain gram negative. The average number of Gardnerella spp. and anaerobic gram negative rods had been quantified. That might appear as small, gram variable pleomorphic coccobacilli. The amount of Mobiluncus morphotypes present had been looked for and quantified .They was often thin, wispy, eyelash-like faintly staining curved gram negative rods. Alternatively they might be much smaller "banana-like" forms with pointed ends. Occasionally, they might stain gram positive. That bacteria were often absent from gram stain smears of persons with other bacterial morphotypes. The relative amounts of each of the three classes were observed morphotypes had been reported. Each morphotypes had been quantified from 0 to 4+ with regard to the numbers of organisms present per oil immersion field as described in Table 1.

Table 1. Calculating Individual Scores Based upon Morphotypes

Numbers of Organisms/Oil Immersion Field

MORPHOTYPE	NON	<1	1-4	5-30	>30
Lactobacilli spp	4	3	2	1	0
Gardnerella &anaerobic GNR	0	1	2	3	4
Mobiluncusspp	0	1	1	2	2

The individual scores for each of the three morphotypes of bacteria; Lactobacilli spp., Gardnerella spp./anaerobic gram negative rods and Mobiluncus spp., respectively should be added together to obtain the total score. The final BV score and the result interpretation are described below

1-3 "Normal vaginal flora"

4-6 "Intermediate"

7-10 Indicative of Bacterial Vaginosis".

The ICSI procedure was performed (4–6) hours after oocyte retrieval to all patients. In preparation for intracytoplasmic sperm injection, the cumulus corona cells are removed by a combined enzymatic and mechanical treatment to denude the oocytes from the cumulus cells. Each oocyte is carefully assessed, noting the presence or absence of germinal vesicle or the first polar body. Only those ova that have been extrude the first polar body (metaphase II) and morphologically intact were suitable for microinjection. Around 12-17 hours after ICSI procedure, fertilization was assessed for evidence of normal fertilization which was defined as the existence of two pronuclei (2PN). Prior to embryo transfer, the developed embryos were graded in accordance with embryo grading system. (10). According to this system the embryo graded as grade1, 2 and 3. Grade 1 embryo with less than 10% fragmentation, stage-specific cell size, no multinucleation.

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Grade 2 embryo with 10–25% fragmentation, stage-specific cell size for majority of cells, no evidence of multinucleation. Grade 3 embryo with severe fragmentation.

Statistical Analysis

Data were collected, summarized, presented and analyzed using three software programs; these are the statistical package for social sciences (SPSS) version 23, Microsoft Office Excel 2010 and MedCalc version 15.8. Dimensional (quantitative) variables were tested for normality distribution using Kolmogorov-Smirnov test; according the index of central tendency was expressed as mean for normally distributed variables and as median for non-normally distributed variables. In addition, index for dispersion was expressed as standard deviation for normally distributed variables and as inter-quartile range (IQR) for non-normally distributed variables, whereas, categorical variables were expressed as number and percentage out of total.

Results

Asymptomatic Bacterial Vaginosis in pregnant and non-pregnant ladies

Rate of asymptomatic bacterial vaginosis as determined by gram stain, whiff test, wet mount and Amsel's criteria was 32.6 % in pregnant women compared to 73.3% in non-pregnant group, being highly significant higher in those women who failed to get pregnant (P<0.001), as shown in table 2.

Table 2.Asymptomatic Bacterial Vaginosis

Test	Pregnant n = 43	Non-pregnant n = 45	P¥
Gram stain, n (%)	14 (32.6)	33 (73.3)	<0.001 HS
Whiff test, n (%)	14 (32.6)	33 (73.3)	<0.001 HS
Wet mount, n (%)	14 (32.6)	33 (73.3)	<0.001 HS
Amsel's criteria, n (%)	14 (32.6)	33 (73.3)	<0.001 HS

n: number of cases; ¥: Chi-square test; HS: highly significant

Discussion:

Rate of infection in pregnant and non-pregnant ladies

Tubal factor infertility is primarily the result of pelvic inflammatory disease (PID) (11). Ghiasi *et al*., found that the prevalence of bacterial vaginosis is 70.34% in infertile women in Qom city which is similar to that found in non-pregnant women enrolled in our study (12).In a study by Liversedge *et al*. which the possible association of BV with infertility in women undergoing IVF treatment was assessed, the prevalence of BV was 25.6% which is lower than our finding (13).

BV could indirectly affect the fertilization. Spandorfer *et al* (14) investigated abnormal vaginal flora and vaginal pro-inflammatory cytokines in women with idiopathic infertility undergoing *in vitro* fertilization. A correlation between bacterial vaginosis, elevated IL-1 β and IL-8, and idiopathic infertility was demonstrated. A recent study conducted in India evaluated the effectiveness of probiotic lactobacilli (Florisia vaginal tablets) on vaginal health and pro-inflammatory cytokines (15).

Conclusion:

Rate of asymptomatic bacterial vaginosis was 32.6 % in pregnant women compared to 73.3% in non- pregnant group, being highly significant higher in those women who failed to get pregnant.

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