The Dynamics of the Vaginal Micro-Ecology During in Vitro Fertilization and Embryo Transfer (IVF-ET) Cycles and its Impact on Pregnancy Outcomes

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Abstract

Background

Abnormal reproductive tract flora may cause infertility, and it may play a key role in the success of assisted reproductive technologies (ART). The obvious short-term changes in estrogen caused by clinical protocols with IVF-ET provide a unique perspective for us to assess the vaginal flora, shifting hormonal condition and investigate the potential associations of the vaginal micro-ecology with cycle outcome of pregnancy. The Vaginal Micro-ecology Evaluation System (VMES) as a tool to analyze the vaginal microbiomes in most areas of China. This study aims to apply the VMES to evaluate the dynamics of vaginal micro-ecology during IVF-ET, and investigate the correlations between vaginal micro-ecology with pregnancy outcome.

Methods

150 patients were enrolled who underwent early follicular phase prolonged protocol IVF-ET due to tubal factors. The VMES is used to evaluate vaginal microbiology indicators of vaginal swabs obtained in different hormonal milieu during the IVF-ET cycle. The pregnancy outcomes were observed, if pregnant.

Results

In our data, the prevalence of bacterial vaginitis (BV) accounts for 3.3%. During IVF procedure, the vaginal microbiome varied across hormonal milieu in some but not all patients. The proportion of BV, and unidentified dysbiosis were increased significantly on the day of human chorionic gonadotropin (HCG) administration. The vaginal micro-ecology on the day of HCG administration correlated with outcome (live birth / no live birth). The multivariable logistic regression model showed that the average age, the duration of infertility, and the vaginal micro-ecology after controlled ovarian hyperstimulation (COH) were associated with the live birth rate.

Conclusion

Our retrospective cohort study suggests that the VEMS has enabled discovery of unidentified dysbiosis shift in the vaginal micro-ecology during IVF-ET therapy. More importantly, the vaginal micro-ecology on the day of HCG administration was significantly associated with the live birth rate.

Plain English Summary

As female reproductive micro-ecological system may affect the success of ART, it is increasingly important to evaluate the dynamics of vaginal micro-ecology during IVF-ET. In this study, the VMES is used to evaluate vaginal microbiology indicators of vaginal swabs obtained in different hormonal milieu during the IVF-ET cycle. During IVF procedure, the vaginal microbiome varied across hormonal milieu in some but not all patients. The vaginal micro-ecology on the day of HCG administration correlated with pregnancy outcome (live birth / no live birth). The average age, the duration of infertility, and the vaginal

micro-ecology after COH were associated with the live birth rate in multivariate logistic regression analysis. In order to improve the efficiency of IVF techniques, we should pay attention to the vaginal micro-ecology on the day of HCG administration.

Introduction

Worldwide, approximately 10–15% of couples have difficulty conceiving spontaneously [1]. ART is the cornerstone of contemporary infertility treatment. Commonly used ART procedures are invasive, costly and do not guarantee a pregnancy. As the success rate of ART is still low over the years, it is increasingly important to understand the hidden causes and to improve the efficiency of IVF techniques. Female reproductive micro-ecological system has been suggested to affect infertility, and it may play a key role in the success of ART, such as embryo implantation and pregnancy [2].

Infections are one of the disrupting problems in this arena. Multiple evidence indicated that infertile patients harbor a differential lower reproductive tract microbiota compared to fertile patients [3, 4]. Therefore, pathological shifts of lower reproductive tract microbiota may be the cause or consequence of conditions in women's infertility. Research has shown that beside the known factors (the woman’s age, duration of subfertility, antral follicle count, and the percentage of motile sperm used) in prediction models, outcome of assisted reproduction might be predicted by the composition of the vaginal microbiota [5].

To perform an IVF-ET cycle, pituitary down-regulation with Gonadotrophin releasing hormone agonist (GnRH-a) is widely used to prevent endogenous luteinizing hormone (LH) surge and spontaneous ovulation, and recruit more follicles in ART. As we all know,during prolonged pituitary down-regulation with full-dose of GnRH-a and controlled COH, the estrogen level in the body rises rapidly from extremely low level in the short term, even exceeding the level during pregnancy. This fluctuation may directly affect the proliferation of vaginal mucosal epithelial cells, vaginal pH, cleanliness and lactobacillus ratio, resulting in vaginal dysbiosis [6]. However, there are few reports on how this short-term estrogen change affects the vaginal flora.

Our study utilized VMES to develop an in-depth and systematic understanding of the composition and ecology of the vagina microbial ecosystem in an IVF population. We aimed to answer the following questions: first, is the change of certain vaginal micro-ecology associated with IVF or intracytoplasmic sperm injection (ICSI) treatment? Second, can the vaginal micro-ecology be used as an independent predictor for IVF or ICSI outcome? Furthermore, the VMES might provide the clinician with valuable information that could increase their understanding of the vaginal micro-ecological status, propensity for infection and treatment regimens for vaginal infectious diseases.

Herein, 150 patients who underwent GnRH-a prolonged protocol IVF-ET were enrolled in the study. The changes of vaginal micro-ecology before and after COH and its correlation with pregnancy outcome were observed and compared,providing new ideas for improving the pregnancy outcome of ART.

Methods

Subjects and procedures

150 patients were enrolled into this study. All patients provided a signed in-formed consent. The mean age of the 150 patients was 30.02 years with a standard deviation of 4.58 years. The Body mass index (BMI) is 18.4-31.6 (22.47±3.15) Kg/m^2. The duration of infertility is 1-10 (3.62±2.13) years.

（1）Inclusion criteria: Patients in childbearing age; patients who firstly underwent GnRH-a prolonged protocol IVF-ET due to tubal factors from January 2018 to December 2018.

（2）Exclusion criteria: Patients with complaints of discharge, itching, burning and dysuria; patients who undergo vaginal douching, or have sexual intercourse within 72 h before specimen acquisition; patients who received systemic antibiotics within 4 weeks; patients who received medication of sexual hormones within 3 months; patients with physical diseases, including cardiovascular, respiratory, endocrine, chronic autoimmune or blood system diseases, etc.

Ovarian stimulation protocol and IVF-ET

All patients in this study was performed GnRH-a prolonged protocol IVF-ET. It is composed of ovarian suppression with a GnRH-a, followed by COH with recombinant and urinary-derived gonadotropins, followed by HCG administration to induce oocyte maturation. Pituitary down-regulation was achieved with a single full-dose injection of 3.75mg GnRH-a (Leuprolide Acetate; Shanghai Livzon Pharmaceutical co., LTD) on day 2 of the menstrual cycle. Successful pituitary down-regulation was confirmed with follicle diameter<8mm, serum estradiol (E_2) <50pg/mL, serum LH<5IU/L, and endometrium thickness<5mm.

Then, COH with recombinant human follicle stimulating hormone (rFSH) ranging from 75 to 300 IU per day would start 32-38 days later. The dosage was determined according to patients' BMI, antral follicular count (AFC) and basal follicle-stimulating hormone (FSH) level. When two leading follicles reached a mean diameter of 18 mm, 6000-8000 IU HCG (Livzon Pharmaceutical Group Inc., China) was used for a trigger.

Transvaginal oocyte retrieval was performed 36-37h after HCG administration. Fertilization was achieved using standard IVF or ICSI. Gametes and embryos were handled separately according to standard laboratory procedures. Embryo quality was analyzed according to the Istanbul consensus workshop on embryo assessment. Fresh embryo transfer was performed on day 3 or day 5 after fertilization. Embryo transfer was determined according to the embryo quality, the thickness and state of endometrium. All embryos were at least good quality (Grade B) with 7-9 cells and less than 10% fragmentation and even symmetry, or high-quality blastocyst.

Luteal phase support was sustained from the oocyte retrieval day and was continued until the day of serum HCG testing. For women with positive HCG data, luteal phase support was continued until 10

weeks of gestation.

Serum E_2 concentration measurements

Blood was drawn, and serum prepared at three time points during each treatment cycle: the baseline; the first day of Gn; the trigger day.

Definition of clinical outcomes

The primary outcome was the live birth rate after fresh embryo transfer. The secondary outcomes include the clinical pregnancy rate, the implantation rate, the biochemical pregnancy rate, the early miscarriage rate.

Biochemical pregnancy was defined with the result of serum β-HCG≥10mIU/ml measured 11 Day after embryo transfer. Clinical pregnancy was defined as detection of gestational sac by transvaginal ultrasound scan 35 days after embryo transfer. The ongoing pregnancy was defined as detection of a viable fetus with fetal heartbeat at 11-12th week of gestation. The early miscarriage was defined as spontaneous abortions within 12 weeks of pregnancy. The live birth rate was classified as delivery of any viable infant after 24 weeks.

Live birth rate (LBR) per fresh transplantation cycle = the number of deliveries/the number of fresh embryo transfer cycles × 100%

Clinical pregnancy rate (CPR) = the number of clinical pregnancy patients/ovulation cycle patients × 100%

Implantation rate (IR) = the number of implantation embryos/the number of transferred embryos × 100%

Biochemical pregnancy rate (BPR) = the number of biochemical pregnancy patients/ the number of fresh embryo transfer patients × 100%

Early miscarriage rate (EMR) = the number of early miscarriage patients/the number of clinical pregnancy patients × 100%

Specimen acquisition and laboratory tests

Vaginal secretion collection

The secretion on the posterior fornix and upper 1/3 segment of the vagina was collected by rotating two sterile long cotton swabs to evaluate the vaginal micro-ecology. The swab 1 was taken on the day of pituitary down-regulation and the swab 2 was taken on the day of HCG administration, respectively. It also required menstruation to be clean for at least 3 days. Patients with LRTI were treated accordingly.

Evaluation of vaginal micro-ecology

Vaginal micro-ecology evaluation system (VMES) includes microscopic detection of flora density, flora diversity, dominant bacterial flora, pathogen, aerobic vaginitis (AV) score for AV, Nugent score for BV and the functional indicators.

(1) Low power microscope was used to observe the presence or absence of trichomonas in wet physiological saline, and AV score was performed at the same time.

(2) After Gram staining of secretion smear, 10×100 times oil microscope was used to check the flora density, diversity and predominant flora, and whether there were budding spores or pseudo hyphae, and Nugent score was carried out.

(A) Flora density: results were recorded as grades I-IV, according to the average number of bacteria in each visual field.

I average number of bacteria is 1-9/field

II average number of bacteria is 10 to 99/field

III average number of bacteria is more than 100/field

IV bacteria aggregate into clusters or densely cover mucosal epithelial cells.

(B) Flora diversity: results were classified into grades I-IV, according to the species number of visible bacteria.

I 1-3 species of visible bacteria

II 4-6 species of visible bacteria;

III 7-9 species of visible bacteria

IV more than 10 species of visible bacteria.

(C) Predominant flora: the largest number of the microorganism species.

(D) Clue cells, budding spores or pseudo hyphae were detected under a microscope by H&E staining.

(3) The five functional indicators: hydrogen peroxide (H_2O_2), sialidase, leukocyte esterase, beta-glucuronidase and coagulase were detected using Aerobic Vaginitis and Bacterial Vaginosis Diagnostic Strip Sets (Beijing Zhong Sheng Jin Yu Diagnostic Technology Co. Ltd).

Diagnostic criteria

- Flora density: “II” and “III” were defined as normal, while “I” and “IV” were defined as abnormal.
- Flora diversity: “II” and “III” were defined as normal, while “I” and “IV” were defined as abnormal.

- Predominant flora: when the dominant bacterial flora was Gram-positive rods, the predominant flora was normal; and other cases were defined as abnormal.
- Functional indicators: When H_2O_2 was positive, it was determined as normal function of Lactobacillus; when the other four items were negative, they were defined as normal.
- AV: A composite AV score of < 3 correspond to “no signs of AV”, 3-4 to “light AV”, 5-6 to “moderate AV”, and any score > 6 to “severe AV” (Table 1).

Table 1. Criteria for the microscopic diagnosis of AV [7]

(×400 magnification, phase contrast microscope)

AV score	LBG	No. of leukocytes	Proportion of toxic leukocytes	Background flora	Proportion of PBC
0	I and IIa	≤10/hpf	None or sporadic	Unremarkable or cytolysis	None or <1%
1	IIb	>10/hpf and ≤10/epithelial cell	≤50% of leukocytes	Small coliform bacilli	≤10%
2	III	>10/epithelial cell	>50% of leukocytes	Cocci or chains	>10%

Lactobacillary grades (LBG) (I) numerous pleiomorph lactobacilli, no other bacteria; (IIa) mixed flora, but predominantly lactobacilli; (IIb) mixed flora, but proportion of lactobacilli severely decreased due to increased number of other bacteria: (III) lactobacilli severely depressed or absent because of overgrowth of other bacteria. hpf: high power field. PBC: parabasal epitheliocytes.

- BV: The diagnostic criteria for BV are as follows: (a) total score 7-10, BV; (b) score 4-6, intermediate BV; sialidase positive as an auxiliary diagnostic indicator (Table 2).

Table 2 The Nugent scoring criteria[8]

Score	*Lactobacillus* morphotypes	*Gardnerella* and *Bacteroides spp.* morphotypes	Curved gram-variable rods
0	4+ >30	0 0	
1	3+ 5~30	1+ <1	1+ <1 or 2+ 1~4
2	2+ 1~4	2+ 1~4	3+ 5~30 or 4+ >30
3	1+ <1	3+ 5~30	
4	0 0	4+ >30	

0, No morphotypes present; 1, <1 morphotype present; 2, 1 to 4 morphotypes present; 3, 5 to 30 morphotypes present; 4, 30 or more morphotypes present.

- Vulvovaginal candidiasis (VVC): microscopic examination of budding yeast or hyphal forms.
- Trichomonas vaginitis (TV): microscopic examination of active trichomonas with numerous white blood cells.
- Normal vaginal micro-ecology (NVM): vaginal flora density grade II-III, flora diversity grade II-III, dominant bacterial flora is lactobacillus, Nugent and AV score ≤3, and absence of pathogens and negative specific enzymes.
- Abnormal vaginal micro-ecology (AVM): any one of vaginal microflora density, diversity, dominant bacteria, inflammatory reaction and vaginal microbial functional indicators is abnormal.

Table 3 Vaginal micro-ecology evaluation system [9]

Items	Normal	Abnormal
Morphological indicators		
Flora density	grades II/III	grades I/IV
Flora diversity	grades II/III	grades I/IV
Predominant flora*	Large Gram-positive rods	Gram-positive cocci Large Gram-negative rods Small Gram-negative rods
Nugent score	1-3	≥4
AV score	<3	≥3
Pathogen	Negative	Fungus budding yeast or hyphal forms and/or trichomonas
Vaginal pH	≥3.8 and <4.5	<3.8 or ≥4.5
Functional indicators		
H_2O_2	Positive	Negative
Enzymes	Negative	Positive

*Large Gram-positive rods (*Lactobacillus);* Gram-positive cocci (*Staphylococcus aureus, Staphylococcus epidermidis*); Gram-negative rods (*Escherichia coli*).

Statistical analysis

Data were analyzed using IBM SPSS 25.0 statistical software. Quantitative data were described as mean ± standard deviation (Mean ± SD) or median. Results were analyzed using *t* tests for comparison

between the study groups and the control group. Pearson $\chi 2$ or Fisher exact test were used for the comparison of proportion, as appropriate. Then, multivariate logistic regression was performed. P <0.05 was considered to indicate statistical significance.

Ethics statement

This study was a retrospective analysis of clinical outcomes, and the Institutional Review Board of the Affiliated Hospital of Qingdao University approved our analysis of the data.

Results

Baseline Characteristics

The swab 1 was taken on the day of pituitary down-regulation and the swab 2 was taken on the day of HCG administration, respectively. For swabs 1, there were no significant differences in age, BMI, type of infertility, duration of infertility, basal FSH, total dosage of Gn used, and duration of stimulation among patients with AVM or NVM (P>0.05). (Table 4)

Table 4. The basal situation and clinical data

Items	NVM	AVM	$t/\chi^2/U$	P
Age ($\bar{X}$±s)	30.62±3.99	31.05±3.46	1.143	0.255
BMI ($\bar{X}$±s)	22.51±3.39	23.17±3.92	0.990	0.324
duration of infertility (median)	3.00	4.00	2275.500	0.096
type of infertility [Primary infertility (%)]	59（52.7%）	41（53.2%）	0.705	0.401
basal FSH(mmol/L,$\bar{X}$±s)	6.98±2.20	7.53±2.58	1.261	0.209
Total dosage of Gn used (IU,$\bar{X}$±s)	2538.20±818.67	2691.78±1029.47	0.934	0.352
Duration of stimulation (d, X±s)	11.48±2.11	11.58±2.52	0.232	0.817

Changes in vaginal micro-ecology during the ovarian stimulation protocol

The proportion of BV, and abnormal flora density/diversity patients evidently increased (P<0.05), while increasing the proportion of patients with intermediate BV, and VVC, but the difference in the latter is not statistically significant (P >0.05).

Table 5. Comparison of VM between swab 1 and swab 2

Items	Swab 1 (%)	Swab 2 (%)	χ^2	*P*
NVM	112（74.7）	77（51.3）	24.596	0.000
VVC	5（3.3）	7（4.7）	0.083	0.774
BV	5（3.3）	14（9.3）	3.765	0.049
Unidentified dysbiosis	28（18.6）	52（34.7）	8.817	0.003
Intermediate BV	9（6.0）	14（9.3）	0.696	0.404
Abnormal dominant flora	4（2.6）	3（2.0）	0.000	1.000
Abnormal flora density/ diversity	15（10.0）	35（23.3）	9.500	0.002

Of the 150 IVF-ET patients, 47 (31.33%) evidenced changes in the VM across all of their swabs. Thus, IVF affects the vaginal micro-ecological environment (*P*<0.01). 36.61% of patients with NVM for swab 1 showed AVM for swab 2, and 84.21% of patients who corrected AVM for swab 1 also showed AVM for swab 2, and the difference was statistically significant. (Table 6)

Table 6. Changes in VM between swabs

Items	NVM of swab 2 (%)	AVM of swab 2 (%)	χ^2	*P*
NVM of swab 1	71 (63.39)	41 (36.61)	24.596	<0.01
AVM of swab 1	6 (15.79)	32 (84.21)		

Correlation between E_2 concentration and vaginal micro-ecology

Serum concentrations of E_2 was measured at baseline and two intervals during the IVF-ET procedure. The E2 level of all patients after downregulation were less than 50pg/mL. The E2 level on the trigger day ranged from 58.58 pg/mL to 3771.38 pg/mL. The VM changed for 73/150 (48.7%) of the IVF-ET patients, while all serum E2 concentrations rose dramatically. The T-Test was employed to make comparisons among patients with NVM and AVM for swab 2. The tests and their p-values were summarized in Table 7. The statistical tests demonstrated no significant difference (*P*=0.345). Thus, it appeared that the VM was not a simple function of circulating E_2 concentration.

Table 7. Correlation between E_2 concentration and VM for swab 2

	E2 level on trigger day($\bar{X}$±s, pg/mL)
NVM for swab 2(n=77)	1684.124±756.887
AVM for swab 2 (n=73)	1565.419±771.625
t	-0.948
P	0.345

Relationship between clinical outcomes and vaginal micro-ecology

Each patient was transferred with one or two good quality embryos only and the number of embryos transferred was comparable between the two groups. The live birth rate (LBR) was 84.42% (65/77) in the NVM group and 45.21% (33/73) in the AVM group, $P<0.01$. The early miscarriage rate (EMR) of the AVM group were also significantly higher than that of the NVM group (30.00% vs 9.43%, $P=0.011$). There were no significant differences in the biochemical pregnancy rate, implantation rate, and clinical pregnancy rate between the two groups ($P>0.05$). (Table 8)

Table 8. Clinical outcomes of IVF-ET patients in two groups

	NVM for swab 2	AVM for swab 2	χ^2	*P*
BPR	74.03%（57/77）	65.75%（48/73）	1.22	0.269
IR	47.95%（70/146）	37.95%（52/137）	2.88	0.090
CPR	68.83%（53/77）	54.79%（40/73）	3.14	0.077
EMR	9.43%（5/53）	30.00%（12/40）	6.46	0.011
LBR	84.42%（65/77）	45.21%（33/73）	25.44	<0.01

Multivariable Logistic Regression Models

We further conducted logistic regression analysis to evaluate whether the vaginal micro-ecology after COH was an independent factor associated with the live birth rate (LBR). Firstly, a binary logistic regression model analysis was carried out to determine the confounding factors. Secondly, the significant variables ($P<0.05$), including age (t=-3.235，$P=0.001$), the duration of infertility (u=1803.500，$P=0.005$), the number of oocytes retrieved (u=3552.500，$P=0.005$), the vaginal micro-ecology before COH ($\chi^2=17.148$，$P=0.000$), and the vaginal micro-ecology after COH (χ2=11.682，$P=0.001$) , identified on the univariate analysis. The multivariable logistic regression model showed that the independent variables predictive of live birth were female age ($P=0.009$), the duration of infertility ($P=0.022$), and the vaginal micro-ecology after COH ($P=0.000$). (Table 9)

Table 9. Logistic Regression Model of vaginal micro-ecology after COH

	β±S	Waldχ^2	df	*P*	OR	OR 95% CI	
						Lower	Upper
Age	-0.147±0.057	6.729	1	0.009	0.864	0.773	0.965
The duration of infertility	-0.207±0.091	5.219	1	0.022	0.813	0.681	0.971
The VM after COH	-1.511±0.411	13.518	1	0.000	0.221	0.099	0.494
Constant	6.374±1.913	11.103	1	0.001	596.175	-	-

Discussion

Several studies indicated that abnormal reproductive tract flora may cause infertility, also may be related to miscarriage, premature rupture of membranes, and premature delivery [10–12]. Since IVF-ET involves transfer of embryos by a catheter through the cervix into the uterus, vaginal and cervical microflora and pathogens and microbial contamination of the catheter tip have been suggested to affect implantation rates and pregnancy outcomes [13–16]. Thus, it is worth considering whether IVF outcomes be influenced by the microbial ecosystem present during infertility treatment.

Vaginal microbiota undergoes important composition fluctuations during women's life, sex hormones playing a key role in this scenario. The vaginal micro-ecological system composed of microbiome, endocrine regulation system, vaginal anatomy and local immune system has been well known [17]. The vaginal microbiome is an intricate and dynamic system [18]. At present, the application of qPCR and 16S rRNA sequencing technology can obtain 107–109 copies of vaginal microflora genes from 1g of vaginal secretions [19]. However, culture-based technologies and 16S rRNA sequencing technology are limited by high cost and low throughput, hence only small numbers of samples have been analyzed, and the depth of sample analysis was not suitable for clinical applications.

When patients complain of abnormal leucorrhea, clinicians should consider the possibility of lower genital tract infections, such as VVC BV, or AV. Sometimes, no pathogens can be detected at all by standard diagnostic techniques [20, 21]. Under these circumstances, what happened to the vagina of these patients? In the case of small shifts in the microbiome, the resulting subtle changes in the local milieu are typically not clinically evident but may remain clinically meaningful. Multicenter epidemiological studies used the VMES as a tool to analyze the vaginal microbiomes in most areas of China [9, 13, 22]. Clinical microbiologist usually identified the vaginal microbiomes through vaginal swab cultures and microscopic examinations to provide a unique "signature" for lower genital tract infections. This tool is composed of morphological and functional indicators. It should be noted that if the functional indicators are inconsistent with the morphological indicators, the morphological indicators

should be taken as reference indicators [13]. VMES could provide a new viewpoint for the comprehensive management of vaginal dysbiosis when infections occur.

Consistent with suppose, the proportion of BV, unidentified dysbiosis (abnormal flora density/diversity, and intermediate BV), and VVC were increased after COH. We were particularly interested in unidentified dysbiosis. Besides BV, we found that abnormal flora density/diversity increased significantly.

Indeed, BV is the most common form of vaginal dysbiosis [23]. BV may be asymptomatic in up to 50% of cases. The incidence of BV is significantly higher in patients with tubal infertility compared with patients with non-tubal infertility[24]. A large heterogeneity was observed among the studies in infertility patients, as the lowest reported prevalence was 4% and the highest prevalence 38% [25, 26]. The early spontaneous miscarriage rate was 28% in BV patients compared with 17% in normal vaginal microbiota patients [27]. Several studies have shown that BV is associated with poorer result, and patients with BV are more likely to experience pregnancy loss after receiving ART [28, 29]. Although BV has received the most attention, abnormal vaginal microbiota is not always BV and other conditions have separate effects on pregnancy outcome. In our data, the prevalence of BV accounts for 3.3%. The five BV patients for swab 1 enrolled in this study are presented in S1. After COH, five patients are also AVM. Moreover, two patients demonstrated non-pregnancy, and three patients had miscarriage. There were fourteen BV patients for swab 2, where twelve patients became from AVM, including BV, intermediate BV, and abnormal flora diversity, etc. Only three patients (21.4%) had a live birth. In addition, two patients became pregnant, but had a miscarriage (S2). Its mechanism has not been fully elucidated so far, and the biologically plausible explanation could be related to intrauterine infection caused by BV-associated bacteria ascending, resulting in chorioamnionitis and inducing uterine contractions [30].

The female genital tract commonly dominated by *Lactobacillus spp.* [31]. The presence of Lactobacillus-dominated microbiome profile has been associated with higher LBR after ART [32, 33], and positively correlated with other reproductive success, namely higher CPR and IR [4, 24, 34]. Pelzer ES et al. [11] have been found that the non-Lactobacillus species,including *Escherichia coli*, *Staphylococcus, Streptococcus, Enterobacteriaceae*, and *G. vaginalis species* associated with poor reproductive outcomes. Different studies have shown that the correlation between AVM and ART results is contradictory. Studies using sequence-based technologies found that AVM had a negative effect on ART. However, studies using culture-based technologies found that AVM was not associated with ART outcome [35]. Hyma et al.[6] showed that the vaginal flora of infertile patients before IVF treatment was not significantly different from that of general gynecological outpatients, but the vaginal flora of some patients changed after receiving different COH treatments, and the abundance of vaginal flora on the day of embryo transfer is related to whether there is a live birth ($P = 0.034$). Singer et al. [28] concluded that women with AVM were approximately 1.4 times less likely to have a successful early pregnancy after an IVF compared with women with normal microbiota.

It is the first study in which the data of VMES used as predictor for IVF outcome. In our current study, the live birth rate (LBR) was declined significantly with AVM on the day of HCG administration. The early

miscarriage rate (EMR) of the AVM group were also significantly higher than that of the NVM group (P = 0.011). There were no significant differences in the biochemical pregnancy rate, implantation rate, and clinical pregnancy rate between the two groups (P > 0.05). As expected, multivariate logistic regression analysis and adjusted marginal means (95% CIs) of the live birth rate also contributed to eliminating the confounding factors. The average age, known as the best predictor of embryo quality, the duration of infertility, and the vaginal micro-ecology after COH were associated with the live birth rate in our regression analysis result. Thus, the data suggests that the vaginal microbiome on the trigger day affects pregnancy outcome.

In the case of the AVM profile, women do not generally suffer from a clinically evident infection. However, our results indicate that women with AVM on the day of HCG administration have a limited chance of success after a fresh embryo transfer. A deep understanding of VM in ART may lead to personalized therapeutic options, such as vaginal administration of antibiotics, pre- or probiotics, aiming at specifically modulation of AVM towards a more normal profile[36]. These women could postpone the fresh ET, freeze the resulting embryos and transfer them later with a NVM profile. Delaying a fresh embryo transfer when couples have a strong wish to have children could lead to discontent. However, Patients may get many benefits, including reducing physical side effects, reducing the economic burden of treatment and mental burden. Our future research will elucidate whether modulation of the vaginal microbiota is possible and whether this may indeed improve outcomes of IVF and IVF-ICSI in patients with an abnormal vaginal micro-ecology profile.

Our research has certain limitations. Firstly, we were not collected the data of vaginal micro-ecology on the day of fresh embryo transfer. Nevertheless, we could still benefit a lot from our data. Secondly, this study was a retrospective design from a single medical center. However, the large sample size, use of a multivariate regression model for a wide array of possible confounding factors, and marginal means of LBR adjusted by protocols rendered the conclusion relatively reliable. Our conclusions warrant further confirmation by larger, multicenter, prospective studies.

Conclusion

Our retrospective cohort study suggests that the VEMS has enabled discovery of unidentified dysbiosis shift in the vaginal micro-ecology during IVF-ET therapy. More importantly, the vaginal micro-ecology on the day of HCG administration was significantly associated with the live birth rate.

Abbreviations

IVF-ET: In vitro fertilization and embryo transfer; ART: Assisted reproductive technologies; VMES: Vaginal micro-ecology evaluation system; BV: Bacterial vaginitis; HCG: Human chorionic gonadotropin; COH: Controlled ovarian hyperstimulation; GnRH-a: Gonadotrophin releasing hormone agonist; LH: Luteinizing hormone; ICSI: Intracytoplasmic sperm injection; BMI: Body mass index; E_2: Estradiol; rFSH: Recombinant human follicle stimulating hormone; AFC: Antral follicular count; FSH: Follicle-stimulating hormone; Gn:

Gonadotropin□LBR: Live birth rate; CPR: Clinical pregnancy rate; IR: Implantation rate; BPR: Biochemical pregnancy rate; EMR: Early miscarriage rate; AV: Aerobic vaginitis; H_2O_2:Hydrogen peroxide; VVC: Vulvovaginal candidiasis; TV: Trichomonas vaginitis; NVM: Normal vaginal micro-ecology; AVM: Abnormal vaginal micro-ecology; OR: Odds-ratio; 95% CI: Confidence interval at 95.

Declarations

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Authors' contributions

All authors contributed to the study conception and design. Xia Liu performed project development. Quan Tian, Yujie Liu, Jiane Liu and Jianru Wu performed material preparation, data collection and analysis. Jianxin Liu, Xiuming Tang, Weihong Hu performed material preparation and data management. Quan Tian wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethical approval and consent to participate All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Consent for publication The manuscript has been read and approved by all authors.

Competing interests The authors report no conflicts of interest in relation to the present study.

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