Genital tract infection and associated factors affect the reproductive outcome in fertile females and females undergoing *in vitro* fertilization

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Abstract. Assisted reproductive techniques including *in vitro* fertilization (IVF) are being used increasingly worldwide and screening for genital tract infections (GTIs) is recommended prior to treatment as their presence may affect the success rate of IVF. The current study aimed to assess the possible associations between GTI-associated factors and reproductive outcome in a group of reproductive age fertile females and infertile females receiving IVF. A total of 111 infertile women enrolled in an IVF programme (Group A) and 104 fertile women (mothers of at least one child; Group B) underwent microbiological screening of vaginal and cervical samples. All samples were cultured using different protocols for aerobic pathogens, bacterial vaginosis (BV), *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Chlamydia trachomatis* and human papilloma virus (HPV). Although each group were comparable in age, more infertile women were >30 years (P=0.0064), had a higher education level (P=0.0001) and were smokers (P=0.007). Only BV (P=0.0013) was more prevalent in Group A. Of the 111 infertile females who were scheduled for IVF, 32 females had a successful pregnancy (Group C) and 79 females exhibited IVF failure (Group D). Tubal factor (P=0.012), estradiol-2 (E2) levels <2,500 pg/ml (P=0.0009) and Mycoplasma infection (P=0.003) were identified to be the strongest predictors of IVF failure. The current study determined certain GTI-associated factors that may contribute to infertility in Greek females of reproductive age as well as other risk factors associated with failure in patients undergoing IVF. Further studies are required to confirm this conclusion.

Introduction

Genital tract infections (GTIs) frequently occur in females of reproductive age and are strongly associated with increased morbidity, including diseases such as urethritis, pelvic inflammatory disease, amniotic fluid infection and preterm deliveries in pregnancy (1,2). Pelvic inflammatory disease involves the infection and inflammation of the upper genital tract (endometrium, fallopian tubes, ovaries and pelvic peritoneum), which may result in infertility, ectopic pregnancy and chronic pelvic pain (3,4). The vaginal microflora is a dynamic ecosystem normally inhabited by lactobacilli. These bacteria support healthy vaginal conditions by maintaining an acidic environment that is inhospitable to other pathogenic microorganisms. *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii* and *Lactobacillus iners* are considered to be the four major vaginal *Lactobacillus* species (5). Many important aspects of women's sexual and reproductive health rely on the protective role of lactobacilli in the vaginal environment. The composition of the vaginal microflora is not static but changes over time in response to various endogenous and exogenous influences. The most common alteration in vaginal microflora

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is a condition named bacterial vaginosis (BV) (6). According to the United States Public Health Service, the incidence of BV is ~30% in females of reproductive age (7). Additionally, the reported incidences of Candida, Mycoplasma and Ureaplasma infections, which are associated with infertility, have increased (8,9). The most common urogenital tract infections associated with infertility are aerobic pathogens, BV, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Chlamydia trachomatis* and human papilloma virus (HPV) (1-4,7-9). The impact of certain bacteria on fertility, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Ureaplasma urealyticum* has been well established (8-10). Furthermore, *Chlamydia trachomatis* infection may cause pelvic inflammatory disease, resulting in chronic pelvic pain, ectopic pregnancy and infertility (10).

Infertility affects ~15% of couples worldwide (11). Human infertility is defined as the inability for a couple to conceive and produce offspring following at least 12 consecutive months of unprotected sexual intercourse (11). Infertility is a complex condition as it may alter the quality of life in couples. In 2010, ~1.9 and ~10.5% of women at reproductive age (20-44 years old) were affected by primary and secondary infertility respectively (11). According to the World Health Organization, the term 'primary infertility' is applied when a woman has not conceived (12). 'Secondary infertility' is the incapability to conceive in a couple who have had at least one previously successful conception (12). There are several causes for this condition, among which urogenital infections serve an important role (13).

In vitro fertilization (IVF) has been utilized worldwide to reduce the rate of infertility. It provides a novel means of preliminary genetic diagnosis and maintenance of fertility, making IVF is the most effective treatment option for couples with multifactorial infertility problems (11,13). Fertility rate, defined as the total number of infants born by a single female in her lifetime, has been estimated to be <1 in 2011 in Greece alone, which is less than the estimated normal rate of >2.1 (14). It has also been observed that ~10-15% of Greek couples experience infertility issues (14). Furthermore, previous studies have indicated that a history of GTI may be associated with IVF failure (11-18), although supporting data remains sparse.

The aim of the current study was to evaluate potential GTI-associated predictors of infertility in Greek females and to assess the factors associated with successful or unsuccessful IVF technique in infertile patients.

Materials and methods

Patients. The current study was prospectively designed and performed at the Department of Microbiology, Aretaieion General Hospital, National and Kapodistrian University of Athens, from January 2012 to December 2017. A total of 125 Greek females of reproductive age (range, 22-45 years; median age, 32), undergoing vaginal/cervical fluid examination as prenatal screening were recruited into the present study. Patients were classified into two groups: 111 infertile females enrolled into an IVF program (group A) and 104 fertile females (having at least one child; Group B; considered to be the control). Epidemiologic data, symptoms and GTIs were compared between groups.

Induction of ovulation and fertilization. In all patients receiving IVF, a long ovulation induction protocol was applied, which included the daily nasal administration of gonadotropin releasing hormone (2 mg/ml; Synarela®; Pfizer, Inc., New York, NY, USA) on the 21st day of the pre-treatment cycle, which included the administration of combined oral contraceptives (0.03 mg ethinyl estradiol and 3 mg drospirenone; each, Yasmin®; Bayer Hellas AG, Athens, Greece) administered for 2 to 4 weeks. Following the downregulation of estradiol-2 by the aforementioned drugs (E2; <50 pg/ml) recombinant follicle stimulating hormone (FSH) was administered subcutaneously (beta-follitropin; Puregon®; Organon International BV, OSS, The Netherlands) in a daily dose of 75-300 IU (depending on the patient's characteristics). When ≥3 follicles of 18 mm in diameter were detected via a trans-vaginal ultrasound, 10,000 IU of human chorionic gonadotropin (hCG) was administered subcutaneously (Pregnyl®; Organon International BV) and oocyte pick-up was performed transvaginally via a thin needle 35-36 h later, under ultrasonography. Following oocyte removal, blood-free follicular fluid samples were centrifuged at 600 x g for 10 min, at 25˚C and supernatants were stored at -80˚C. The *in vitro* fertilization of oocytes was accomplished using the spermatozoa of the patient's respective husband and all embryo transfers were performed on day 3 with a Wallace catheter under ultrasound guidance.

Patient characteristics. Patients in group A were assessed for IVF success and for potential predictors of failure. Patients that successfully became pregnant (Group C; 32 patients) were compared with patients unable to achieve pregnancy (Group D; 79 patients) in regards to the type of pathogen isolated In patients scheduled for IVF, basic characteristics including E2 levels, the number of oocytes retrieved, the number of oocytes fertilized and the number of transferred embryos were recorded. Serum E2 levels (centrifuged at 600 x g for 10 min at 25˚C) were measured using the Abbott Architect i-1000SR auto-analyzer (Abbott Pharmaceutical Co., Ltd., Lake Bluff, IL, USA; sensitivity, 10 pg/ml; intra-assay and inter-assay coefficients of variability, 5.5 and 6.7%, respectively). Additionally, factors contributing to infertility including endometriosis, male factor (male infertility) or tubal factor (anatomical disorders) were reported.

Vaginal discharge examination. In all patients, specimens of vaginal discharge were obtained from the posterior fornix for microscopy, amine testing, and gram staining at 25˚C for 5 min. Solution A (90% Crystal violet; 20 ml of 95% Ethanol) and Solution B (0.8 g Ammonium oxalate with 80 ml Distilled water) were mixed to obtain a crystal violet staining reagent. Samples were stored for 24 h at 25˚C and filtered through paper prior to use. At the completion of the Gram Stain, gram-negative bacteria stained pink/red and gram-positive bacteria stained blue/purple. Patients from which vaginal samples were not collected were excluded from the current study. Amine testing ('whiff' test) was performed by adding a drop of potassium hydroxide 10% to vaginal discharge samples. The test was considered positive if the typical fishy odor was produced. A gram-stained smear was examined via light microscopy (magnification, x1,000) and the presence of yeast and leukocytes was also determined. Specimens for yeast culture, were incubated at 37˚C for 24-48 h and placed in

modified Stuart's medium and plated on Sabouraud dextrose agar (both from BioMerieux, Marcy l'Etoile, France). Vaginal samples were cultured for aerobic pathogens at 37˚C for 24-48 h and the automated system VITEK2 (BioMerieux) was used to identify the pathogens present.

BV diagnosis. BV was diagnosed via the Nugent scoring system (19). This method assesses the quantity of three different bacterial morphotypes: Large gram-positive rods (representing lactobacilli), small gram-variable rods (representing *Gardnerella* and *Bacteroides*/*Prevotella* species) and curved rods (representing the *Mobiluncus* species). On the basis of these results, samples were assigned a score from 0 to 10, with 1-3 being considered as normal, 4-6 being considered as intermediate bacterial count (where patients receive chemoprophylaxis treatment) and 7-10 being considered as BV. BV diagnosis was further verified using the Amsel criteria (20), which includes the presence of at least three of the following four findings: homogeneous vaginal discharge, vaginal pH >4.5, 'fishy' odour on addition of potassium hydroxide to vaginal fluid (positive 'whiff test') and the presence of clue cells in microscopy of wet preparation (20). When each test (Nugent scoring system with Amsel criteria) was positive for either the presence of fungus, trichomonas vaginalis or bacterial vaginosis, the diagnosis of BV was confirmed.

Aerobic vaginitis (AV) diagnosis. The presence of AV was determined using an 'AV' score (21). The score was calculated using high-power field light microscopy (magnification, x1,000) to assess the presence or absence of healthy lactobacilli, the number of leukocytes, the number of leukocytes with toxic granulation, the type of vaginal flora and the number of parabasal epithelial cells. An 'AV' score of <3 corresponds to no signs of AV, 3-4 to light AV, 5-6 to moderate AV and >6 to severe AV. Candidiasis was diagnosed in symptomatic patients when yeast culture in modified Stuart's medium and plated on Sabouraud dextrose agar was positive for any Candida species and no other pathogen was detected.

Ureaplasma urealyticum and Mycoplasma hominis detection. *Ureaplasma urealyticum* and *Mycoplasma hominis* were detected using the Mycoplasma IST2 kit (BioMerieux). The kit contained strips that provide information on the presence or absence of *Ureaplasma urealyticum* and *Mycoplasma hominis*, and also provides additional information on susceptibility to different antibiotics. Samples were vortexed rapidly at 3,500 rpm at 25˚C for 1 min and they were used (in their liquid form) to rehydrate the lyophilized growth medium provided in the kit. The Mycoplasma strips were then inoculated with 100 μl of the rehydrated growth medium overlaid with two drops of oil, then incubated at 37˚C and observed for colour changes. The results were interpreted following 24 and 48 h of incubation at 37˚C according to the manufacturer's protocol. Samples with titres $\geq 10^4$ CCU/ml were considered positive. Finally, *Chlamydia trachomatis* was detected using the COBAS AMPLICOR test kit (cat. no. 04341341 190; Roche Diagnostics, Indianapolis, IN, USA).

Human papilloma virus (HPV), cytomegalovirus (CMV), Toxoplasma, Rubella, HIV, Hepatitis B virus and Hepatitis C virus (HCV) detection. HPV was detected using the LINEAR ARRAY HPV Genotyping Test (Roche Diagnostics). This method detects 21 HPV genotypes of high risk (including 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73 and 82) and 16 HPV genotypes of low risk (6, 11, 40, 42, 54, 55, 61, 62, 64, 71, 72, 81, 83, 84, IS39 and CP6108) in cervical cells from each group preserved in PreservCyt® solution, (ThinPrep® PreservCyt® Solution; Hologic, Inc., Marlborough, MA, USA) according to the manufacturer's protocol. Additionally, anti-CMV, anti-Toxoplasma gondii and anti-Rubella IgG and IgM antibodies were measured in serum using a chemiluminescent microparticle immunoassay (Architect i-1000SR; Abbott Pharmaceutical Co., Ltd.). For the diagnosis of HIV-1/HIV-2 infection, the Architect HIV Ag/Ab Combo assay, a chemiluminescent microparticle immunoassay was utilized (Abbott Pharmaceutical Co., Ltd.). Additionally, serum Hepatitis B surface antigen, anti-HBV and anti-HCV titers were determined via an enzyme immunoassay using the ARCHITECT platform (Abbott Pharmaceutical Co., Ltd.) according to the manufacturer's protocol.

Statistical analysis. The incidence of demographics and symptoms were reported in all study groups (group A vs. group B) and subgroups of women receiving IVF (group C vs. group D). The type and number of symptoms as well as the type and number of pathogens were compared among all groups. To compare nominal data between two groups, the Chi-Square or Fisher test were utilized, while for all data expressed in numeric format, a Student's t-test was used. $P<0.05$ was considered to indicate a statistically significant difference. All P-values were calculated based on absolute values. Subgroup analysis was performed to determine potential predictors of fertility or IVF success. Furthermore to exclude any confounding factors and calculate the relative risk (RR) for the two groups, multivariate analysis using logistic regression was used. All statistical analyses were performed using IBM SPSS v.24 software (IBM Corp., Armonk, NY, USA).

Ethical approval. Finally, the protocol for this research project was approved by a suitably constituted Ethics Committee of Aretaieion Hospital within which the work was undertaken and it conforms to the provisions of the Declaration of Helsinki (as revised in Tokyo 2004).

Results

Study group characteristics. A total of 215 females undergoing prenatal cervical/vaginal fluid testing in Aretaieion hospital (within a period of 5 years) were included in the current study. These comprised 111 infertile females (Group A) scheduled for IVF and 104 fertile females (Group B). The mean age of patients did not differ significantly between the two groups, although a greater number of females from Group A were >30 years of age compared with Group B (P=0.0064). Additionally, fertile females had a mean number of 1.23 children. Group A had a higher education level compared with Group B (P=0.0001), as well as a higher smoking prevalence (P=0.007). However, no significant differences in symptoms were observed between groups. In Group A, only BV (P=0.0013) was significantly more prevalent. Furthermore, infertile females possessed

Table I. Demographics and type of pathogen present in fertile and infertile female patients.

Parameter	Overall (n=215)	Fertile (n=104)	Infertile (n=111)	P-value
Age (years)	31.17	30.32	31.98	NS
Age ≤30 years	105	61	44	0.006
Number of children per female	0.56	1.23	0	<0.0001
Higher education	69	11	58	0.0001
Smoking	76	27	49	0.007
Symptomatic of urinary-tract infection	66	29	37	NS
Symptoms				
Irregular discharge	66	29	37	NS
Dysuria	5	2	3	NS
Dyspareunia	9	7	2	NS
Pruritus	32	16	16	NS
Pain	15	5	10	NS
Type of infection				
Genital mycoplasmas	32	11	21	NS
Aerobic vaginitis	22	8	14	NS
Bacterial vaginosis	59	18	41	0.001
Candida species	13	5	8	NS
CMV	20	12	8	NS
HPV	41	22	19	NS
HCV	0	0	0	NS
HIV	0	0	0	NS
HBV	55	23	32	NS
Toxoplasma	55	26	29	NS
Rubella	184	88	96	NS
Chlamydia trachomatis	16	7	9	NS
Decreased *Lactobacillus* species	179	79	100	0.006
1 pathogen	34	9	25	0.008
2 pathogens	1	0	1	NS
>2 pathogens	35	25	10	0.003

NS, not significant; CMV, cytomegalovirus; HPV, human papilloma virus; HCV, hepatitis C; HIV, human immunodeficiency virus; HBV, hepatitis B virus; higher education, college education.

Table II. Characteristics and type of infection present in females undergoing *in vitro* fertilization.

Factor	Overall (n=111)	Pregnant (n=32)	Non-pregnant (n=79)	P-value
Estradiol-2 levels (pg/ml)	2234.38± 1614.06	2853.75± 1393.58	1983.49± 1619.4	0.007
Retrieved oocytes (mean)	4.9	6.13	6.3	NS
Fertilized oocytes (mean)	4.26	5.31	4.41	0.002
Embryos (mean)	2.56	2.63	3.84	0.004
Transferred embryos (mean)	2.26	2.63	2.22	0.008
Male factor infertility (n)	17	11	6	NS
Tubal factor infertility (n)	39	6	6	0.0009
Endometriosis (n)	9	2	33	0.028
Ovulation disorder (n)	12	4	7	NS
Unexplained infertility (n)	34	9	8	NS
Vaginal discharge samples				
1 pathogen	1	0	25	NS
2 pathogens	10	4	1	NS
>2 pathogens	100	28	6	NS
Mycoplasma species	21	2	72	NS
Aerobic vaginitis	14	4	19	0.03
Bacterial vaginosis	41	10	10	NS
Candida species	8	4	31	NS
CMV	8	3	4	NS
HPV	19	8	5	NS
HCV	0	0	11	NS
HIV	0	0	0	NS
HBV	0	0	0	NS
Toxoplasma	29	10	0	NS
Rubella	96	27	19	NS
Chlamydia trachomatis	9	3	69	NS

NS, not significant; CMV, cytomegalovirus; HPV, human papilloma virus; HCV, hepatitis C; HIV, human immunodeficiency virus; HBV, hepatitis B virus.

>2 pathogens more frequently when compared with fertile females (P=0.006; Table I).

Pregnancy outcome. Of the 111 infertile females enrolled in the IVF program, 32 had a successful pregnancy (Group C) and the remaining 79 patients did not (Group D). The mean E2 levels for all patients undergoing IVF was 2234.38±1614.06 pg/ml, with E2 levels being higher in Group C compared with Group D (2853.75±1393.58 vs. 1983.49±1619.40 pg/ml; P=0.007). Furthermore, the number of fertilized oocytes (P=0.002) and the number of resulting embryos (P=0.004) was higher in Group C compared with group D. Male factor was more prevalent in Group C (P=0.0009) while tubal factor was more prevalent in Group D (P=0.028). Other factors, including endometriosis or ovulation disorder, did not significantly differ between the two groups. Of the pathogens examined in the current study, only Mycoplasma was detected more frequently in Group D (P=0.03; Table II).

Multivariate analysis. The results of multivariate analysis revealed that a higher education [relative risk(RR)=2.31; 95% confidence interval (CI), 1.825-2.939; P<0.0001] was the strongest predictor associated with infertility. Other factors associated with infertility were as following: An age of >30 years, smoking, the presence of decreased lactobacillus species, the presence of >2 pathogens and the presence of BV (Table III). Furthermore, tubal factor (RR=1.32; 95% CI,

Table III. Multiregression analysis for potential risk factors associated with infertility or *in vitro* fertilization failure.

Risk factors	Relative risk (95% CI)	P-value
Infertility		
Age >30 years	1.45 (1.109-1.905)	0.007
Higher education	2.31 (1.825-2.939)	<0.0001
Smoking	1.45 (1.127-1.855)	0.004
Decreased *Lactobacillus* species	1.55 (1.201-1.994)	0.0007
>2 pathogens	1.83 (1.099-2.043)	0.02
Bacterial vaginosis	1.55 (1.215-1.974)	0.0004
IVF failure		
Tubal factor	1.32 (1.064-1.649)	0.012
Estradiol-2 levels <4,000 pg/ml	1.48 (0.868-2.539)	NS
Estradiol-2 levels <3,000 pg/ml	1.49 (1.007-2.213)	0.046
Estradiol-2 levels <2,500 pg/ml	1.78 (1.265-2.501)	0.0009
>2 pathogens	1.13 (0.712-1.798)	NS
Genital mycoplasmas	1.36 (1.110-1.660)	0.003

Odds ratio and 95% confidence intervals were evaluated. The statistical significance level was defined as P<0.05. NS, not significant; higher education, college education; CI, confidence interval.

1.064-1.649; P=0.012), E2 levels <2,500 pg/ml (RR=1.78; 95% CI, 1.265-2.501; P=0.0009) and Mycoplasma infection (RR=1.36; 95% CI, 1.110-1.660; P=0.003) were determined to be the strongest predictors for IVF failure (Table III).

Discussion

The current study determined that infertility was associated with specific epidemiologic factors and certain genital tract pathogens in Greek females of reproductive age. IVF failure in infertile females was also associated with tubal factor infertility and the presence of Mycoplasma species in their genital tract. Furthermore, increased age and a higher level of education were also associated with increased infertility in the current study. This is congruent with the results of previous studies, which have reported lower fertility rates in older females (22,23). This may be due to females going to college or university and pursuing a professional career, thus becoming pregnant at an older age where oocyte number and quality has decreased, which is particularly evident after reaching the mid 30s (24). Despite the fact that higher education is associated with higher age, they were examined independently as predisposing factors of infertility in the logistic regression analysis model of the current study, which may require further investigation. Furthermore, the instance of higher education may be attributed to infertility by mechanisms other than age alone, including (25) i) less interest in having large families so that they may provide better for fewer children; ii) the better health status of educated women and of their children result in the higher survival rate of children and a reduction of the need for more children; iii) educated women are using contraceptives more often.

In the current study, smoking was revealed to decrease fertility. It has been indicated that >30% of females at reproductive age are smokers (26). In an older meta-analysis, Augood *et al* (27) determined that women who smoked had a significantly higher odds ratio of infertility (OR 1.60; 95% CI, 1.34-1.91), in comparison to non-smokers. In addition, previous studies have indicated that low ovarian reserves, low hormone levels and tubal dysfunction may be possible mechanisms by which smoking affects fertility rate in females (28-30).

Certain factors including BV and the presence of >2 pathogens were associated with low fertility in the present study. A previous study has demonstrated that BV is associated with pelvic inflammatory disease, preterm birth and infertility (31). Particularly in the case of a combined infection, the burden of pathogens could easily cause an imbalance in the cervical/uterine microbiome or dysbiosis predisposing to cervical disease or even infertility, according to a previous study (32). Although the current study did not detect an association between infertility and other pathogens, previous studies have identified a strong association of Mycoplasma and chlamydia co-infection with infertility (33,34). Finally, the current study identified that a decreased concentration of *Lactobacillus* species (AV score, <3) was associated with low fertility. This concurs with a previous study, where a significantly lower Lactobacilli concentration was identified in patients with BV (35). The protective potential of lactobacilli is based on their ability to regulate normal pH and inhibit the growth of vaginal microorganisms known to cause infection and adverse effects (36). A previous study has indicated that a non-Lactobacillus-dominated microbiota is associated with a significant decrease in endometrial receptivity and pregnancy rates (37).

The current study also identified that tubal factor, low E2 levels and the presence of Mycoplasma were strongly associated with IVF failure. In a previous study by Kawwass *et al* (38), tubal factor infertility was associated with a higher miscarriage rate, preterm birth and a low birth weight risk compared with male factor infertility. Additionally, low E2 levels on the day of hCG administration have also been associated with adverse IVF outcomes (39). Joo *et al* (40) reported an E2 concentration-dependant effect on IVF outcomes, concluding that the optimal range of serum E2 levels in women is age-dependent: 3,000-4,000 pg/ml for women <38 years and 2,000-3,000 pg/ml for women ≥38 years. However, a meta-analysis has revealed that only female age, duration of subfertility, basal FSH levels and the number of oocytes are predictors of pregnancy following IVF (41).

Considering the effect of genital tract pathogens on IVF outcomes, co-infection or infection due to genital Mycoplasmas were associated with IVF failure in the current study. Although different viruses have been previously associated with IVF failure (42,43), no such association was identified in the cohort of the present study. This may be due to the increased administration of the HPV vaccination in Greece during the recent years (44). Furthermore, Mycoplasma infections and co-infections have been strongly associated with infertility in the general population, although data specifically on patients undergoing IVF remains limited (45). Therefore, to the best of our knowledge, this is one of the first studies to assess the associations of several pathogens with IVF failure. Most presented evidence in previous literature regarding infertility in such patients is indirect, with Mycoplasma infections

causing the production of several pro-inflammatory cytokines (including interleukin-1, 12 and 18, tumor necrosis factor-α and interferon-γ), which may provide a mechanistic connection between inflammatory stimulus and *in vivo* fertilization or embryonic growth following IVF (46).

In conclusion, the current study identified a positive association between BV and infertility in a large-scale cohort of reproductive-age Greek females as well as a strong association between genital Mycoplasmas and IVF failure. Additionally, other predicting factors were determined to be associated with a negative IVF outcome. These results may result in improved management strategies for such patients to reduce infertility and failure rates in patients undergoing IVF. However, further studies with a larger number of participants and more specific criteria may be required to support the present findings, the outcome of which may improve IVF.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DM developed, planned and supervised the project, and wrote the manuscript. GD performed data entry and evaluation and wrote the manuscript. SB developed and supervised the current study and wrote the manuscript. PA, RK, EK, IP, PP, AK and NA collected relevant literature. AP performed the whole statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The current study was approved by the Medical Ethics Committee of Aretaieion Hospital, University of Athens (Greece). All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, and later versions. Informed consent or substitute for it was obtained from all patients prior to enrolment.

Patient consent for publication

All patients participating in the present study were informed in detail and agreed to the publication of associated data (and any accompanying images) as appropriate, fully respecting their anonymity and medical ethics. The patients' consent forms from the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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