Abstract. – OBJECTIVE: We evaluated the prevalence of cervicovaginal bacteria, group B Streptococcus (GBS), Gardnerella vaginalis (GV), Candida spp., Chlamydia trachomatis (CT), Mycoplasma hominis (MH) and Ureaplasma urealyticum (UU) in pregnant women with and without diabetes mellitus (DM).

PATIENTS AND METHODS: Cervicovaginal swabs were gathered from 473 pregnant patients divided into 127 diabetic and 346 non-diabetic. The results were correlated to gestational age, parity and glycemic control.

RESULTS: A higher prevalence of MH/UU (p=0.012) was found in the diabetic patients. After the 28th week of pregnancy, the prevalence for all investigated microorganisms appeared similar except for MH/UU (p=0.014). In multigravida, there were statistical differences between two groups in testing for Bacteria (p=0.015) and for MH/UU (p=0.037). The diabetic condition correlated to the state of multigravida in cases positive for Candida spp. (p=0.049) and in those testing positive for at least one microorganism (p=0.043). Pregnant with a blood glucose > 92 have twice the risk of being positive to a single microbiological test than those with better glycemic control.

CONCLUSIONS: The higher prevalence of MH/UU after the 28th week can be explained with the physiologically reduced insulin tolerance characteristic of this gestational period. Among the diabetic testing positive to Candida spp. the statistically significant association was observed only in multigravida condition. These data suggest that diabetic multigravida women are at increased risk for Candida spp. infection in relation to the improper glycemic control.

Key Words
- Gestational diabetes mellitus
- Cervicovaginal microorganisms
- Cervicovaginal microbiota
- Bacterial vaginosis

Introduction

The cervicovaginal niche is a complex ecosystem of microbial communities with a dynamic balance. Studies conducted with molecular biology, microscopy and a culture-based method have documented frequent and rapid fluctuation in the composition of vaginal communities1-6. The composition of this flora is determined by different factors such as age, hormonal stage, the different phases of menstrual cycle, sexual activity, the use of hormonal contraception, the use of products for intimate hygiene, pregnancy stage, various pathologies (infections, endocrinopathy, etc.) and treatments (immunosuppressive, immunomodulatory, antibiotic therapies, etc). Since menarche, under normal circumstances, there appears to be a predominance of Lactobacilli that serve as protection against infections sustained by pathogenic organisms7-10.

Local immunity, along with the vaginal microbiome, is an essential part of the defense against infection.

During pregnancy, the amount of estrogen in circulation escalates causing an increased amount of glycogen in the vaginal epithelium, generally with flora having 90% Lactobacillus and both aerobic and anaerobic germs. These germs originate partially from intestinal and skin contamination, and partially belong to the vagina’s own saprophytic flora. Eventual inflammatory responses or infections are caused by an alteration in the quality or quantity of the bacterial components previously described11-13.

Epidemiological studies have shown that pregnancies with bacterial and fungal infections are at higher risk of maternal and neonatal adverse events14-22.
Systematic pathologies during pregnancy can also affect the vaginal bacterial flora, thus increasing ascendant infections. Among these pathologies, diabetes mellitus is considered an independent risk factor for various vaginal and/or rectal colonization and infection, particularly by Candida spp. and Streptococcus group B.

A higher level of estrogen in pregnancy and more glycogen in cervicovaginal mucosa make it easier for the yeast to grow. While progesterone suppresses the anti-Candida activity of neutrophils, estrogen reduces the ability of vaginal epithelial cells to control the growth of Candida albicans and decreases the level of immunoglobulins in a vaginal niche, leading to increased vulnerability of pregnant women to Candida spp. colonization (Figure 1).

The mollicutes Mycoplasma hominis and Ureaplasma urealyticum, common saprophyte of vaginal niche, lacking a cell wall and cytoplasmic membrane, are considered minimal bacterial cell prototypes with reduced metabolic abilities and different energy-generating pathways that may colonize and infect mucosal epithelium (Figure 2).

Cervical and vaginal epithelium colonization of UU and MH is frequent in sexually mature asymptomatic women and vary according to age, socioeconomic status, and sexual activity. They

**Figure 1.** Cervicovaginal Candida infection in diabetic patients.
Cervicovaginal microorganisms in pregnant diabetic and non-diabetic women

are implicated in different genital infections and diseases such as cervicitis, pelvis inflammatory disease, infertility, adverse obstetrical outcomes (premature delivery, premature rupture of membranes) and also in neonatal infections. Not sufficient data exist regarding the presence of different bacterial flora in pregnancies affected by diabetes. For this reason, we conducted an observational study to evaluate if there is a different prevalence of cervicovaginal microorganisms in two gestational conditions: normal and diabetic.

Patients and Methods

This multicenter observational cohort study was conducted between November 2011 and July 2013 in three different centers: 1) the Outpatient Gynecology and Obstetrics in collaboration with the Outpatient Diabetes at Sant’Andrea Hospital; 2) the Center for cure and prevention of diabetes mellitus, Tor Vergata University of Rome and 3) the Diabetes Unit, Saint Camillo Forlanini Hospital, Rome, Italy. The study was reviewed and approved by the Institutional Review Board.

Figure 2. Cervicovaginal Mycoplasma infection in diabetic patients.
Cervicovaginal swabs were collected from 473 pregnant patients. Clinical and anamnestic records concerning each woman enrolled were taken into consideration. To be eligible, pregnant women had to be free of subjective complaints, clinical symptoms of vaginosis, vaginal infection or vaginal bleeding. The patients all presented with a singleton pregnancy and with amniocorial membranes intact. They also could not have received oral or local antimicrobial treatments within the four weeks prior to enrollment. None of the patients took corticosteroids or any other immunomodulant drugs, and they all resulted negative for HIV. All participants gave informed consent.

The study population was divided into two groups. The first group presented 127 pregnant patients with diabetes mellitus: gestational diabetes (103 cases), pre-gestational diabetes (24 cases: 14 with type 1 diabetes, 10 with type 2) with an age average of 34.91 years (± 5.02). The second group presented 346 non-diabetic pregnant patients with an average age of 32.84 years (± 4.69). The BMI of the diabetic and non-diabetic group was 28.5 (±6.8) and 23.6 (±4.1) respectively.

Furthermore, in two subgroups of diabetic women, we considered metabolic control parameters: in 72 cases the glycated hemoglobin level evaluated during the last month before the vaginal swabbing, and in another 45 cases the capillary glycemia results obtained on the day of the microbiological sampling.

Considering the gestational age, the population was divided into those within the first 28 weeks of pregnancy (≤ 28) and those past the 28th week (> 28), this week being relevant for screening and diagnosis of gestational diabetes. The parity was divided into primigravida and multigravida (≥ second pregnancy).

To diagnose the cervicovaginal infection, genital specimens for different microorganisms (Bacteria, GBS, GV, Candida spp., CT, MH, and UU) were performed according to the medical request that did not include all the germs in each case. All the swabs were processed in our microbiology laboratory.

To detect the presence of Candida spp. and Bacteria like Enterococcus spp., Staphylococcus spp., Escherichia coli, Klebsiella pneumonia, the vaginal swabs were plated on Blood agar (BioMerieux, Capronne, France), MaConkey agar (BioMerieux, Capronne, France), CHROMagar Candida medium (Beckton Dickinson, Heidelberg, Germany) and cultivated under aerobic conditioning. Also, anaerobic culturing was performed on Columbia CAN agar (BioMerieux, Capronne, France) and Gardnerella Selective Agar with 5% human blood (Beckton Dickinson, Heidelberg, Germany) by using an anaerobic box. All plates were incubated at 37°C for 24-48 hours before the examination. The finding of bacterial colonies was followed by standard microscopic and biochemical tests for isolation and final identification. A search for pathogenic germ was conducted only where there were no Lactobacilli, and an inflammatory state certified by a high number of leukocyte were present.

Participants were tested for CT using the COBAS Amplicor PCR (Roche Applied Science, Mannheim, Germany) and TIB MOLBIOL (Syntheselabor GmbH, Berlin, Germany) and the assay was performed exactly as described in the manufacturer’s package inserts.

For genital mycoplasmas infections (UU and MH), a Mycoplasma IST2 kit (BioMerieux, Capronne, France) was used for biochemical determination. The test was performed according to the manufacturer’s instructions. This test allows identification of mycoplasma pathogens within 48 hours and an estimation of the amount of bacteria to differentiate between colonization and infection. We considered the test positive when the amount of MH/UU was >10⁷ cells. It also provides additional information on antibiotic susceptibility.

**Statistical Analysis**

A comparison was made between the results obtained from the group of diabetic and those from the non-diabetic pregnant group using a χ²-test. The variables of the two groups considered were analyzed statistically comparing the average values with the Student’s t-test and the Mann-Whitney test for variables not paired. Values under 0.05 were considered significant. Calculating the odds ratio with a confidence interval of 95%, a univariate analysis was done for metabolic factors associated with diabetes as well as for the association between possible risk factors like a gestational period and the occurrence infection. Values of p ≤ 0.05 were considered statistically significant. All statistical data was obtained using the statistical program STATA 12 SE (StataCorp LLC, College Station, TX, USA).

**Results**

The analysis of the characteristics of the 473 pregnant patients studied evidences that the diabetic women are of a more advanced average age (p=0.001) and have a higher average BMI (p<0.001).
The results of the microbiologic research are shown in Tables I, II and III.

Comparing the two groups of women examined, a considerably higher prevalence of bacterial infection was found in the diabetic patients by MH/UU (χ²-test, p = 0.012). Contrarily, the percentage of CT infection showed to be higher in the non-diabetic women (χ²-test, p = 0.053). No statistically significant differences were found between the two groups when tested for Bacteria (χ²-test, p = 0.092), GV (χ²-test, p = 0.975), Candida spp. (χ²-test, p = 0.870), GBS (χ²-test, p = 0.791) and for positive results to at least one test versus negative to all tests (χ²-test, p = 0.226) (Table I).

The prevalence of the microorganisms obtained by cervicovaginal swabs from the diabetic population, divided into pre-gestational diabetes and gestational diabetes, did not show statistically significant differences between the two groups of participants. This regarding a positive result to at least one test versus negative results to all tests (χ²-test, p = 0.31), and considering the single isolated microorganisms: Bacteria (χ²-test, p = 0.474), GV (χ²-test, p = 0.62), Candida spp. (χ²-test, p = 0.679), GBS (χ²-test, p = 0.457), CT (χ²-test, p = 0.383) and MH/UU (χ²-test, p = 0.561).

Table II compares the results of swabs from diabetic and non-diabetic women within the gestational period ≤ 28 weeks and > 28 weeks. Among the cases in the first period, the two metabolic subgroups presented similar results for Bacteria (χ²-test, p = 0.113), GV (χ²-test, p = 1), Candida spp. (χ²-test, p = 0.471), GBS (χ²-test, p = 0.891), CT (χ²-test, p = 0.143) and MH/UU (χ²-test, p = 0.586) while the number of diabetic women versus non-diabetic resulting positive to at least one cervicovaginal test was statistically higher (χ²-test, p = 0.046). After the 28th weeks, the percentage of cases testing positive for a

Table I. Prevalence of microorganisms isolated in pregnant diabetic and non-diabetic women.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Diabetic /tot no. (%)</th>
<th>Non-diabetic /tot no. (%)</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria*</td>
<td>15/111 (13.5%)</td>
<td>20/299 (8%)</td>
<td>0.092</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>9/49 (28.8%)</td>
<td>41/221 (16.8%)</td>
<td>0.975</td>
</tr>
<tr>
<td>Candida spp</td>
<td>24/117 (20.5%)</td>
<td>58/293 (29.8%)</td>
<td>0.870</td>
</tr>
<tr>
<td>Group B</td>
<td>16/114 (14%)</td>
<td>47/312 (15.1%)</td>
<td>0.791</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>3/111 (2.7%)</td>
<td>23/286 (8%)</td>
<td>0.053</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>21/112 (18.8%)</td>
<td>28/290 (9.7%)</td>
<td>0.012</td>
</tr>
<tr>
<td>Positive to at least one vs negative to all</td>
<td>70/127 (55.1%)</td>
<td>169/346 (48.8%)</td>
<td>0.226</td>
</tr>
</tbody>
</table>

*Bacteria: Gram positive, Gram negative

The prevalence of the microorganisms obtained by cervicovaginal swabs from the diabetic population, divided into pre-gestational diabetes and gestational diabetes, did not show statistically significant differences between the two groups of participants. This regarding a positive result to at least one test versus negative results to all tests (χ²-test, p = 0.31), and considering the single isolated microorganisms: Bacteria (χ²-test, p = 0.474), GV (χ²-test, p = 0.62), Candida spp. (χ²-test, p = 0.679), GBS (χ²-test, p = 0.457), CT (χ²-test, p = 0.383) and MH/UU (χ²-test, p = 0.561).

Table II compares the results of swabs from diabetic and non-diabetic women within the gestational period ≤ 28 weeks and > 28 weeks. Among the cases in the first period, the two metabolic subgroups presented similar results for Bacteria (χ²-test, p = 0.113), GV (χ²-test, p = 1), Candida spp. (χ²-test, p = 0.471), GBS (χ²-test, p = 0.891), CT (χ²-test, p = 0.143) and MH/UU (χ²-test, p = 0.586) while the number of diabetic women versus non-diabetic resulting positive to at least one cervicovaginal test was statistically higher (χ²-test, p = 0.046). After the 28th weeks, the percentage of cases testing positive for a

Table II. Prevalence of microorganisms isolated in pregnant diabetic and non-diabetic women ≤ 28 weeks and > 28 weeks.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>≤ 28 weeks</th>
<th>&gt; 28 weeks</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria*</td>
<td>10/34 (29.4%)</td>
<td>7/47 (14.9%)</td>
<td>0.113</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>4/10 (40%)</td>
<td>8/20 (40%)</td>
<td>1</td>
</tr>
<tr>
<td>Candida spp</td>
<td>11/37 (29.8%)</td>
<td>15/43 (34.9%)</td>
<td>0.471</td>
</tr>
<tr>
<td>Group B</td>
<td>2/35 (5.7%)</td>
<td>2/40 (5%)</td>
<td>0.891</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>5/77 (13.1%)</td>
<td>17/252 (16.4%)</td>
<td>0.614</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>1/33 (3%)</td>
<td>5/40 (12.5%)</td>
<td>0.143</td>
</tr>
<tr>
<td>MH/UU</td>
<td>6/33 (18.2%)</td>
<td>6/44 (13.6%)</td>
<td>0.586</td>
</tr>
<tr>
<td>Positive to at least one versus negative to all</td>
<td>28/39 (72%)</td>
<td>29/54 (53.7%)</td>
<td>0.046</td>
</tr>
</tbody>
</table>

*Bacteria: Gram positive, Gram negative
single germ among the diabetic and non-diabetic women appeared relatively similar except for MH/UU, which was higher in the diabetic patients ($\chi^2$-test, $p=0.014$).

Irrespective of metabolic conditions, the univariate analysis showed a correlation between the gestational period ≤ 28 weeks and a positive result to at least one microbiological test with OR (95% IC) of 1.72 (1.0-2.69), since the values were statistically significant ($\chi^2$-test, $p=0.021$).

With reference to parity and positive test for a single microorganism, a comparison was made between the diabetic and the non-diabetic patients. This showed that only in multigravida women there were statistically relevant differences between diabetic and non-diabetic in testing for Bacteria ($\chi^2$-test, $p=0.015$) and for MH/UU ($\chi^2$-test, $p=0.037$). Even in this context, CT was more prevalent in non-diabetic ($\chi^2$-test, $p=0.023$). The same was not found for primigravida women (Table III).

Furthermore, in diabetic women subgroup either positive or negative to a single test and positive or negative to at least one microbiological test, the diabetic condition and parity appeared correlated (Table IIIa). Statistically, this correlation was significant in cases testing positive for Candida spp. ($p=0.049$) and for at least one microorganism ($p=0.043$). Whereas among the patients who tested negative to a single test, the diabetic condition was linked in a significant way to a multigravida state only in cases resulting negative to GBS ($p=0.047$).

In 45 diabetic study women, the capillary glucose measurements taken into consideration were those got at the moment of the microbiological swabbing. The median blood glucose level resulted in 92 mg/dl with a range of 65-165 mg/dl. Whereas in 72 women the levels of glycated hemoglobin considered were those obtained within one month from sample taking. The glycated hemoglobin median value observed was 5.2 (range 3.3-7.9).

The median glycemic value showed no statistic differences between the 25 women testing positive for at least one microbe (median BG=98 mg/dl, range 74-130) and the 20 women resulting negative on all tests (median BG=91 mg/dl, range 65-165) (test Mann Whitney=1.247, $p=0.212$). Likewise, no statistic difference was found among the glycated hemoglobin values: the women resulting positive to at least one test (n 40) compared to those testing negative to all tests (n 32) showed to have an average of HbA1c, accordingly 5.35 (range 3.4-7.2%) and 5.27 (range 3.3-7.9%) (Student’s $t$-test, $p=0.353$).

### Table III. Correlation between parity and prevalence of microorganisms isolated in pregnant diabetic and non-diabetic women.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Parity</th>
<th>Diabetic/total no. (%)</th>
<th>Non-diabetic/total no. (%)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Primigravida</td>
<td>6/52 (11.5%)</td>
<td>17/159 (11%)</td>
<td>$p=0.865$</td>
</tr>
<tr>
<td></td>
<td>Multigravida*</td>
<td>9/59 (15%)</td>
<td>7/140 (5%)</td>
<td>$p=0.015$</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>Primigravida</td>
<td>4/21 (19%)</td>
<td>22/118 (19%)</td>
<td>$p=0.965$</td>
</tr>
<tr>
<td></td>
<td>Multigravida*</td>
<td>5/27 (19%)</td>
<td>19/103 (18%)</td>
<td>$p=0.993$</td>
</tr>
<tr>
<td>Candida spp</td>
<td>Primigravida</td>
<td>9/52 (17%)</td>
<td>31/159 (20%)</td>
<td>$p=0.727$</td>
</tr>
<tr>
<td></td>
<td>Multigravida*</td>
<td>15/65 (23%)</td>
<td>27/134 (20%)</td>
<td>$p=0.635$</td>
</tr>
<tr>
<td>Group B Streptococcus</td>
<td>Primigravida</td>
<td>8/51 (16%)</td>
<td>29/168 (17%)</td>
<td>$p=0.793$</td>
</tr>
<tr>
<td></td>
<td>Multigravida*</td>
<td>8/63 (13%)</td>
<td>18/144 (13%)</td>
<td>$p=0.968$</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>Primigravida</td>
<td>3/53 (6%)</td>
<td>12/155 (8%)</td>
<td>$p=0.613$</td>
</tr>
<tr>
<td></td>
<td>Multigravida*</td>
<td>0/58 (0%)</td>
<td>11/131 (8%)</td>
<td>$p=0.023$</td>
</tr>
<tr>
<td>Mycoplasma hominis/ Ureaplasma urealyticum</td>
<td>Primigravida</td>
<td>11/52 (21%)</td>
<td>19/160 (12%)</td>
<td>$p=0.095$</td>
</tr>
<tr>
<td></td>
<td>Multigravida*</td>
<td>10/60 (17%)</td>
<td>9/130 (7%)</td>
<td>$p=0.037$</td>
</tr>
</tbody>
</table>

*≥ second pregnancy
Table IV shows the correlation between these clinical variables and positive results of cervicovaginal tests. Pregnant women having a glycemic value higher than the median >92 mg/dl were approximately two times more at risk of resulting positive to at least one microbiological test than the group of women having a glycemic level <92 mg/dl (OR 1.9, 95% CI: 0.497-7.46).

**Discussion**

An epidemic of obesity and diabetes mellitus is affecting growing numbers of women in their childbearing years and increasing their risk of obstetric complications. The scientific literature states that with aging, as well as in the second part of pregnancy, women show higher glycemic levels and a greater appearance of glucose intolerance. In line with the above-mentioned literature, in our study, a BMI >25 is associated with diabetic condition.27-29.

It is “widely accepted” that the incidence of infections is greater in diabetes mellitus patients. The higher frequency of cervicovaginal infections is, indeed, associated with a multitude of factors related to the elevated glucose concentrations in the vaginal mucosa, which favors immune dysfunction such as damage to the neutrophil function, depression of the antioxidant system, and humoral immunity, etc.32-35. In the present study, the prevalence in isolation of Gram negative and
Gramp positive bacteria like *Enterococcus* spp., *Staphylococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae* did not result higher in the diabetic compared to the non-diabetic subjects also when considered in the two different gestational age.

No statistically significant differences were found between the two groups concerning the isolation of *GBS*, according to the work of Reimer et al\textsuperscript{44} and Piper et al\textsuperscript{45}, which claim that metabolic changes in gestational diabetes do not alter the level of vaginal colonization by *GBS*, contrary to what other authors reported\textsuperscript{41-43,46-47}. In our experience, there were no differences in the prevalence of *Candida* spp. between the two groups of pregnant women when globally considered and subdivided according to gestational age (≤ 28 or > 28 weeks). The fact is related to a proper glycemic control. Our data contradicts previous publications that observed a higher prevalence of *Candida* spp. in diabetic women\textsuperscript{35-40}, especially if pregnant. Furthermore, Nowakowska et al\textsuperscript{35} reported that the risk of vaginal mycoses in pregnant women with diabetes mellitus type I was more than four times higher than in controls, and in pregnancies with gestational diabetes mellitus the infection rate appeared nearly two times higher than in controls. Our results do not meet these conclusions. In accordance with Nowakowska et al\textsuperscript{35} and Parveen et al\textsuperscript{58}, in the present study, the prevalence of fungi in diabetic pregnant women was not influenced by gestational ages.

Instead, among diabetic patients testing positive for *Candida* spp., the infection of *Candida spp.* is more frequent in multigravida women compared to primigravida ones. Even Parveen et al\textsuperscript{58} observed an increased ratio of vaginal Candida in diabetic and multigravida pregnancies, suggesting that these women require routine screening for vaginal candidiasis regardless of symptomatic status. The explanation for this association should be found in metabolic and immunologic modifications caused by diabetes mellitus on the one hand and in hormonal and anatomic changes caused by multiparity on the other hand. Therefore, the result of this hormonal-immunologic-anatomic-metabolic synergy in diabetic multigravida leads to the local immune system dysfunction and to cervicovaginal microbe changes in the presence of endocervical epithelium eversion and ectropion typical for the childbearing age. However, not only the changes in the host, but also in the microbes resident should be considered. Fungi isolated from pregnant diabetic women, compared to those isolated from healthy women present lower activity of

\begin{table}
\centering
\caption{Correlation between clinic and metabolic characteristics in diabetic pregnant women resulting positive to at least one microbiological test.}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Clinical variables} & \textbf{Positive to at least one test} & \textbf{OR (95% IC)} & \textbf{\(\chi^2\)} \\
& \textbf{Yes} & \textbf{No} & \textbf{} \\
& \textbf{no.} & \textbf{no.} & \textbf{} \\
& \textbf{(%)} & \textbf{(%)} & \textbf{} \\
\hline
\textbf{Diabetes Type} & & & \textbf{} \\
Non-diabetic & 169 & 177 & 1.415 & \textbf{p=0.121} \\
& (48.8\%) & (51.2\%) & (0.89-2.257) & \textbf{} \\
GDM & 59 & 44 & 0.727 & \textbf{p=0.560} \\
& (57.3\%) & (42.7\%) & (0.2-2.435) & \textbf{} \\
Type 1 & 6 & 8 & 0.978 & \textbf{p=0.973} \\
& (42.8\%) & (57.2\%) & (0.222-4.31) & \textbf{} \\
Type 2 & 5 & 5 & & \textbf{} \\
& (50\%) & (50\%) & & \textbf{} \\
\hline
\textbf{Glycemia (mg/dl)} & & & \textbf{} \\
< 92 & 11 & 12 & 1.909 & \textbf{p=0.286} \\
& (47.8\%) & (52.2\%) & (0.497-7.46) & \textbf{} \\
\geq 92 & 14 & 8 & & \textbf{} \\
& (63.6\%) & (36.4\%) & & \textbf{} \\
\hline
\textbf{Glycated hemoglobin} [%] & & & \textbf{} \\
\leq 5.2 & 18 & 13 & 1.222 & \textbf{p=0.709} \\
& (58\%) & (42\%) & (0.457-3.27) & \textbf{} \\
> 5.2 & 22 & 19 & & \textbf{} \\
& (53.7\%) & (46.3\%) & & \textbf{} \\
\hline
\end{tabular}
\end{table}
both alkaline (ALP) and acid phosphatase (ACP)\textsuperscript{32}. In diabetic patients, the development of mycoses is not simply associated with hyperglycemia and high vaginal glucose levels, but is rather a complex result of different concomitant events such as glucose metabolism in fungal cells in which extracellular hydrolytic enzymes could take part.

There was no difference in the prevalence of Candida spp. isolates in pregnant women with GDM versus those with pre-pregnancy DM, contrary to the findings in the study by De Leon et al\textsuperscript{38}.

In accordance with Nowakowska et al\textsuperscript{36}, no correlation was found between metabolic parameters (HbA1c and glycemia) and positive results to at least one microbiological test, which can be explained by multiple factors that participate in the infective and immune mechanisms and that do not always have an immediate or fast cause-effect action.

The positive rate for CT observed in our study is in line with previous literature\textsuperscript{59-61}, which reports a prevalence in pregnancy that can reach 15\% in relation to age, the population, and the method used. Particularly interesting is the result regarding a higher positive rate among the group of non-diabetic women, with a difference that is close to the statistical significance. This could be attributed to the awareness that diabetic patients have to be at higher risk of encountering infection also through sexual transmission. Furthermore, when comparing diabetic pregnant patients with non-diabetic in relation to parity and positivity for CT, a statistically significant difference was found only among the multigravida. This finding confirms the higher prevalence previously observed in pregnancies of non-diabetic women, and it underlines the possible relation between reproductive performance and sexually transmitted infections in multigravida pregnancies.

It is known that GV is one of the bacteria responsible for bacterial vaginosis, even if the presence “per se” of this organism is not sufficient for the development of this polymicrobial syndrome, a condition considered as a risk factor for pregnancy complications\textsuperscript{62-64}. In the vagina the microbic population live in a delicate mutualistic relationship with the host and play an important role in the defense against the infection by opportunistic pathogens. With the advent of the molecular methods using PCR amplification of the bacterial 16S rRNA gene, five major types of the vaginal microbiota called “Community state types” were identified. Four of these are characterized by the presence of the Lactobacillus crispatus (CST-I), Lactobacillus iners (CST-III), Lactobacillus gasseri (CST-II) or Lactobacillus jensenii (CST-V). The CST-IV, instead, does not contain a significant quantity of the Lactobacillus and is composed of a polymicrobial complex of strict and facultative anaerobes including species of the genera Gardnerella, Atopobium, Mobiluncus, Prevotella and other taxa in the order Clostridiales\textsuperscript{65,66}. In this study, the percentage of positive cultures for GV was in line with data reported in the literature and, additionally, there were not discovered different isolates between the two groups of pregnant women in different gestational ages. Therefore, diabetes does not appear to increase the risk of GV infection. This data supports the role played by the vaginal flora’s balance also in the case of multiple changes that occur during the different gestational ages confirming the limitations of a culture test in the investigation of this equilibrium.

A higher rate of infection by MH/UU was reported in our study in diabetic women compared to the reference group, with a statistically significant difference. It is interesting to notice that, differently from what was observed for all the other microorganisms considered, the gestational period appeared to affect the colonization process of these bacteria. In fact, after the 28\textsuperscript{th} week, we found a statistically significant greater vaginal infection.

Furthermore, only in the group of multigravida pregnancies, there was a significant difference between diabetic and non-diabetic concerning MH/UU, a fact not encountered in primigravida women. These findings are in contrast with the results of Castellano-Gonzales et al\textsuperscript{49}, who reported the highest positive percentages of Mycoplasmas in primigravida and during the second gestational trimester.

The colonization of the genital tract by Mycoplasmas is under the influence of hormones that reach high levels during pregnancy: those dependent on estradiol, such as Ureaplasmas and M. hominis, are urea, arginine, or arginine/glucose metabolizing while those dependent on progesterone, such as M. genitalium, are glucose-metabolizing\textsuperscript{64}. Our findings suggest that the MH/UU infection, estradiol-requiring Mycoplasmas, observed after the 28\textsuperscript{th} week could be related to the selective receptor mechanism and synergistic high level of estrogens. We speculate that the multiparity condition in diabetic pregnant women could have an additional role along with other cofactors such as hormonal, metabolic and immunological in the balance of this biologic niche.
There are at least 3 essential limitations of this study: a) this is mainly an observational design that used cultivation-dependent techniques for bacteria, fungi, and MH/UU evaluation. Molecular methods using PCR amplification of the bacterial 16S rRNA gene may have led to different results of our study; b) we considered only two Mycoplasmas (MH/UU). The further use of PCR amplification may have led to different results in pregnant diabetic population; c) we did not obtain Gram stain for bacterial vaginosis data (Nugent’s score) for all the pregnant women and correlate it to the increased prevalence of genital Mycoplasmas in the diabetic population.

Conclusions

At present, the dynamics of bacterial species resident in the vaginal tracts of healthy pregnant and the modifications which occur in diabetic gestation are not well defined and still need longitudinal investigation. Understanding how all these bacterial species interact with each other and the host vaginal epithelium is essential for a more complete understanding of the vaginal health of both pregnant and non-pregnant women.

While the normal microbiota may prevent infection and colonization of the host and the spread of microorganisms related to urogenital infections, including those responsible for bacterial vaginosis, fungal, viral, protozoal and aerobic bacterial vaginitis, the disturbed vaginal flora, especially when asymptomatic, could cause female and male diseases, infertility and be primarily associated with an adverse pregnancy outcome, as well as maternal and fetal morbidity.

Much of our knowledge about the composition of the cervicovaginal microbial flora comes from qualitative descriptive studies using cultivation-dependent techniques that failed to properly characterize vaginal microbiological communities and data obtained by different methods are not comparable. In the last decade, genes amplification of cultivation-independent techniques has enabled a better knowledge of the composition of the vaginal ecosystem-microbial phylogeny. On the other hand, these methods are limited by their tendency to sample only the most prevalent bacteria in a community, likely accounting for a low-abundance or minority species to be overlooked. Despite their limitations, cultivation studies remain an important part of vaginal microbiology and will need to be used in combination with cultivation-independent techniques.

Obviously, the availability of molecular methods for the study of microbiome may have led to different results of our study. The results we observed in this study could point to the need of genetic methods to clarify the questions it poses.

Over the last few years, a more aggressive and diversified clinical and diagnostic approach to gestational diabetes has accounted for a better selection of diabetic pregnancies, improving the obstetric management and outcome.

In conclusion, our data does not support a more prevalent vaginal infection by Candida spp., GBS and by GV in diabetic women. However, it shows that proper glycemic control is the main objective in diabetic pregnancies for the purpose of both preventing a colonization and infection of various microorganisms and insuring better mother and fetal outcome.

Indeed we observed that the gestational diabetes mellitus (GDM) condition correlates to a higher risk of resulting positive to a vaginal swab test. This observation is in global agreement with data reported in the literature.

Among the vaginal microbiological tests here considered, a positive result to at least one is more prevalent at ≤ 28 weeks, when microbiological screening is not generally performed.

Published antenatal care guidelines recommend microbiological screening in pregnancy and search generally for third trimester GBS, while bacterial vaginosis and other infections are investigated only in symptomatic or in those at risk.

In future, clinical practice guidelines should include screening for cervicovaginal infection in each diabetic pregnancy (pre-gestational and gestational) or only in cases of uncontrolled DM? How many hyperglycemic events per day, per week, or per month “do microbes need” to alter the vaginal microbiota-host equilibrium? We still need many answers from clinical research.

During pregnancy, it would be beneficial to undertake prospective studies using different but comparable diagnostic methods of microbiome and viromia, agreed timing for sampling during the gestational stages and for the obstetrical outcome. Only in this way will the scientific community be able to understand if it is the case to implement the current recommendations for microbiological screening, for example extending the search to different Mycoplasmas even through a PCR or to specific bacterial cultures, and/or anticipate the screening for women with pre-gestational and gestational diabetes. Perhaps even anticipate screenings to a pre-conception period with a true cost-benefit convenience.
Cervicovaginal microorganisms in pregnant diabetic and non-diabetic women

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Conflict of interest
The authors declare no conflicts of interest.
66) Fiedorck DN. Molecular methods to describe the spectrum and dynamics of the vaginal microbiota. Anaerobe 2011; 17: 191-195.
72) www.siditalia.it.
73) www.aemmedi.it.