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Vaginal microbiota in pregnant women with inflammatory rheumatic and inflammatory bowel disease: A matched case-control study

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Abstract

Background: Rheumatic diseases and vaginal infections both increase the risk of preterm birth. It is unclear whether pregnant women with rheumatic disease are more likely to experience vaginal infections, which might potentially accumulate modifiable risk factors.

Objective: In this study, we sought to evaluate the vaginal microbiota of pregnant women with inflammatory rheumatic and inflammatory bowel disease.

Methods: A total of 539 asymptomatic women with singleton pregnancy were routinely screened for an abnormal vaginal microbiota between 10 + 0 and 16 + 0 gestational weeks. Vaginal smears were Gram-stained and microscopically analysed. Those with inflammatory diseases (with or without immunomodulatory therapy) were assigned to the case group and matched in a 1:3 ratio to healthy pregnant controls.

Results: Overall, an abnormal vaginal microbiota occurred more frequently among women of the case group, compared with those of the control group (33.8% vs 15.6%; 95% CI: 1.78–4.27, $p < .001$). In particular, *Candida* colonisation (22.3% vs 9.2%; 95% CI: 1.69–4.75, $p < .001$), but also bacterial vaginosis (14.9% vs 7.2%; 95% CI: 1.25–4.1, $p = .006$), occurred more often in the case than in the control group. No significant difference was found with regard to the occurrence of an abnormal vaginal microbiota between subgroups with and without immunomodulatory treatment (37.0% vs 27.1%; 95% CI: 0.29–1.35, $p = .232$).

Conclusion: Pregnant women with inflammatory rheumatic and inflammatory bowel disease are at risk for bacterial vaginosis and *Candida* colonisation, which might pose a risk for preterm birth. Prospective studies are needed to further evaluate the influence of autoimmune conditions and immunosuppressive therapy on the vaginal microbiota.

KEYWORDS

Candida albicans, candidosis, immunomodulatory therapy, inflammatory bowel disease, inflammatory rheumatic disease, microbiota, preterm birth, vulvovaginal infection

1 | INTRODUCTION

Pregnant women with systemic inflammatory autoimmune diseases are known to have an increased risk of adverse pregnancy outcomes, both of the mother and the offspring. In particular, those with high activity of the underlying inflammatory autoimmune disease have a higher risk for miscarriage, preterm birth (PTB), stillbirth, low birthweight, as well as caesarean section, preeclampsia and gestational diabetes.[1–3] Pregnancy outcomes of women with stable disease are similar to those of the general obstetric population, but adequate treatment with immunomodulatory agents is often required to manage disease activity during pregnancy.[1,3,4]

Apart from the risk for the pregnancy itself, inflammatory autoimmune disease poses a risk for various infectious conditions, such as pneumonia, influenza, fungal diseases, as well as infections with human papillomavirus (HPV) and herpes simplex.[5] The fact that these women have an a priori increased risk to experience PTB when being compared to healthy women,[1–4] is even aggravated by the altered hormonal and immune situation during pregnancy which might, at least in part, increase their likelihood for infections.[6] According to our previous work, 28% of pregnant women showed asymptomatic vaginal infections at antenatal screening smears, whereof candidosis was found in 13.2%, bacterial vaginosis (BV) in 8.7% and trichomoniasis in 0.7% of the cases.[7] This is of particular importance, considering the fact that vaginal infections are responsible for up to 40% of PTB, which is still the leading cause of neonatal mortality and morbidity.[8,9]

Little is yet known about the vaginal microbiota of women with inflammatory autoimmune conditions. Only, a few studies have investigated their vaginal microbiota; some have reported a link between immunosuppression, autoimmune disease and vaginal infections,[10–13] and others have demonstrated contradictory results.[14] However, no data yet exist about the incidence of vaginal infections in rheumatic patients during pregnancy. In order to avoid infection-related complications and prevent an accumulation of risk factors for PTB, it is indeed important to further evaluate the comparative risk of an abnormal vaginal microbiota that could lead to vaginal infections in these women.

In the present study, we sought to evaluate the vaginal microbiota of pregnant women with inflammatory rheumatic and inflammatory bowel disease, comparing it to a group of matched healthy controls. Our aim was to assess whether pregnant women with inflammatory rheumatic or inflammatory bowel diseases are at risk for an abnormal vaginal microbiota, including both bacterial and fungal diseases. Since infections are a modifiable risk factor for PTB, their early detection through consequent screening and treatment might be recommended for these women.

2 | PATIENTS AND METHODS

2.1 | Patients and groups

This study was approved by the ethical review committee of the Medical University of Vienna (No: EK 1128/2018). We included retrospectively collected data from all women with inflammatory rheumatic diseases (ie systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, seronegative spondyloarthritis, mixed connective tissue disease, undifferentiated connective tissue disease, Sjögren's syndrome, etc) and inflammatory bowel diseases (ie ulcerative colitis, Crohn's disease), who routinely presented with singleton pregnancy at the Department of Obstetrics and Gynecology, Medical University of Vienna (Vienna, Austria), between January 2004 and November 2017. Data of these women were assigned to the case group and stratified between women who received immunomodulatory therapy during pregnancy (DWT) and those who did not receive any immunomodulatory therapy (DNT). The control group consisted of healthy women with singleton pregnancies without any chronic maternal conditions (eg diabetes mellitus, multiple sclerosis, neoplasms, etc). Inclusion criteria for cases and controls were as follows: registration for a planned delivery between 10 + 0 and 16 + 0 gestational weeks, maternal age ≥18 years, singleton pregnancy. The diagnosis of an autoimmune disease through a specialist (ie rheumatologist or gastroenterologist) was considered mandatory for all women who were assigned to the case group; those with missing or inconclusive data were excluded. Cases and controls were matched in a 1:3 ratio, according to maternal age at delivery and body mass index (BMI).

2.2 | Outcome measures

The vaginal microbiota at routine infection screening served as the primary outcome variable. The null hypothesis stated that there was no difference in the occurrence of an abnormal vaginal microbiota (AVM) between pregnant women with inflammatory rheumatic diseases and/or inflammatory bowel disease and healthy controls. AVM was defined as the occurrence of BV with a Nugent score of 7–10,[15] and/or the occurrence of *Candida* species, and/or *Trichomonas vaginalis* on the vaginal screening smear. Women with a normal or intermediate microbiota (ie Nugent scores 0–3 or 4–6) with the absence of *Candida* spp. and *Trichomonas vaginalis* were considered as normal. Secondary outcome variables included gestational age at delivery, maternal BMI, neonatal birthweight, mode of delivery, Apgar score and umbilical cord arterial pH value. In addition, we assessed foetal and maternal outcomes, as well as pregnancy complications among different inflammatory disorders. Immunomodulatory therapy was defined as treatment with corticosteroids, biologic disease-modifying antirheumatic drugs (DMARDs) and/or non-biologic DMARDs, given for a minimum of four weeks throughout the pregnancy. PTB was defined as delivery prior to 36 + 6 weeks of gestation. Stillbirth was defined as the delivery of an infant with an Apgar score of 0/0/0 or an infant that had died in utero.

2.3 | Infection screening

Asymptomatic women who presented for a planned delivery at our centre were routinely screened at their first visit as part of our routine protocol. Vaginal smears were assessed with sterile swabs from the posterior vaginal fornix and lateral vaginal wall, then applied on

TABLE 1 Maternal characteristics of 539 cases and controls

Variable	Case group *N* (%) M ± SD	Control group *N* (%) M ± SD	*p*-value	DWT subgroup *N* (%) M ± SD	DNT subgroup *N* (%) M ± SD	*p*-value
Participants	148 (27.5)	391 (72.5)		100 (18.6)	48 (8.9)	
Age at delivery	31.1 ± 5.2	31.4 ± 4.8	.571	31.1 ± 5.2	31.1 ± 5.0	.970
BMI	24.3 ± 5.0	24.1 ± 3.9	.521	23.9 ± 4.4	25.2 ± 5.9	.161
Gravidity	2.4 ± 1.6	2.64 ± 1.7	.155	2.4 ± 1.7	2.6 ± 1.5	.417
Parity	1.8 ± 1.1	2.21 ± 1.2	.001	1.7 ± 1.1	1.9 ± 1.0	.544
Primiparous	73 (49.3)	140 (35.8)	.004	53 (53.0)	20 (41.7)	.197
Multiparous	75 (50.7)	251 (64.2)	.004	47 (47.0)	28 (58.3)	.197
Smoker	24 (16.3)	61 (15.7)	.855	20 (20.0)	4 (8.5)	.143
Non-smoker	123 (83.7)	328 (84.3)	.855	80 (80.0)	43 (91.5)	.143
Alcohol abuse	1 (0.7)	0 (0.0)	.103	0 (0.0)	1 (2.1)	.079
Previous PTB	18 (12.2)	49 (12.5)	.908	14 (14.0)	4 (8.3)	.323

Note: All values except number and *p*-value are mean ± standard deviation.
Abbreviations: BMI, body mass index; DNT, disease without therapy; DWT, disease with therapy; N, number; PTB, preterm birth.

a microscope slide for direct visualisation, Gram-stained and analysed in our in-house laboratory by trained and experienced staff. The vaginal microbiota was graded using the Nugent scoring system, differentiating between (a) Gram-positive rods (*Lactobacillus* spp.), (b) small Gram-negative rods (*Bacteroides* spp.), small Gram-variable rods (*Gardnerella vaginalis*) and (c) curved rods (*Mobiluncus* spp.). The Nugent score was then calculated by the sum of a + b + c, whereas a score of 0–3 was considered as normal, 4–6 as intermediate and 7–10 as BV.[15] For internal validation, the vaginal microbiota was also determined by the degrees of purity, as described and modified by Robert Schroeder in 1921.[16] The presence or absence of *Candida* spp. (ie hyphes, blastospores or chlamydospores) and *Trichomonas vaginalis* was assessed independently from the Nugent scoring system. All women who were screened were asymptomatic without clinical signs and symptoms that are pathognomic for ongoing vaginal infections. Those with BV on Gram-stained smears received 2% clindamycin vaginal cream for 6 days, or oral clindamycin (0.3 g) twice daily for 7 days in case of a recurrent disease. Women with *Candida* colonisation received local clotrimazole (0.1 g) for 6 days, and those with trichomoniasis received local metronidazole (0.5 g) for 7 days. Antibiotic treatments were always followed by supplementation of vaginally applied lactobacilli for 6 days to rebuild the microbiota, as well as by consequent follow-up smears.

2.4 | Data collection

Eligible cases with inflammatory rheumatic or inflammatory bowel diseases were identified using the obstetric documentation software (ViewPoint® Fetal Database, version 5.6.9.17) using the following search terms in the diagnosis field for ambulatory visits: “rheu*”, “rheuma”, “rheumatoid arthritis”, “arthritis”, “SLE”, “lupus”, “Morbus Still”, “colitis ulcerosa” and “crohn”. In addition, we searched for specific medications, such as synthetic and biological disease-modifying antirheumatic drugs, glucocorticoids. The selection of cases, as described by the inclusion criteria, as well as the manual search for detailed demographic and clinical data, was performed by VM. Data search and quality were reviewed by KR. For data protection, patients were given consecutive study numbers and data were anonymised.

2.5 | Statistical analysis

Graphs, diagrams and univariate statistical analyses were performed using the SPSS statistical software package version 23.0 (IBM). For metric variables, we used mean ± standard deviation (M ± SD). Categorical variables are presented as numbers (absolute frequencies and percentages). Significance ($p < .05$) was tested using a two-sided chi-squared test. We accounted for multiplicity by applying the Bonferroni correction to the resulting *p*-values when appropriate. Multivariate logistic regression was tested to assess relative independent associations of AVM on the dependent outcome of preterm birth.

3 | RESULTS

3.1 | Study population and demographic data

The data of 539 women were eligible for the analyses, whereof 148 were considered as cases with inflammatory rheumatic or inflammatory bowel diseases, being matched in a 1:3 ratio to 391 healthy controls. The 148 cases consisted of 100 cases (67.6%) with immunomodulatory therapy during pregnancy (DWT) and 48 cases (32.4%) without immunomodulatory therapy during pregnancy

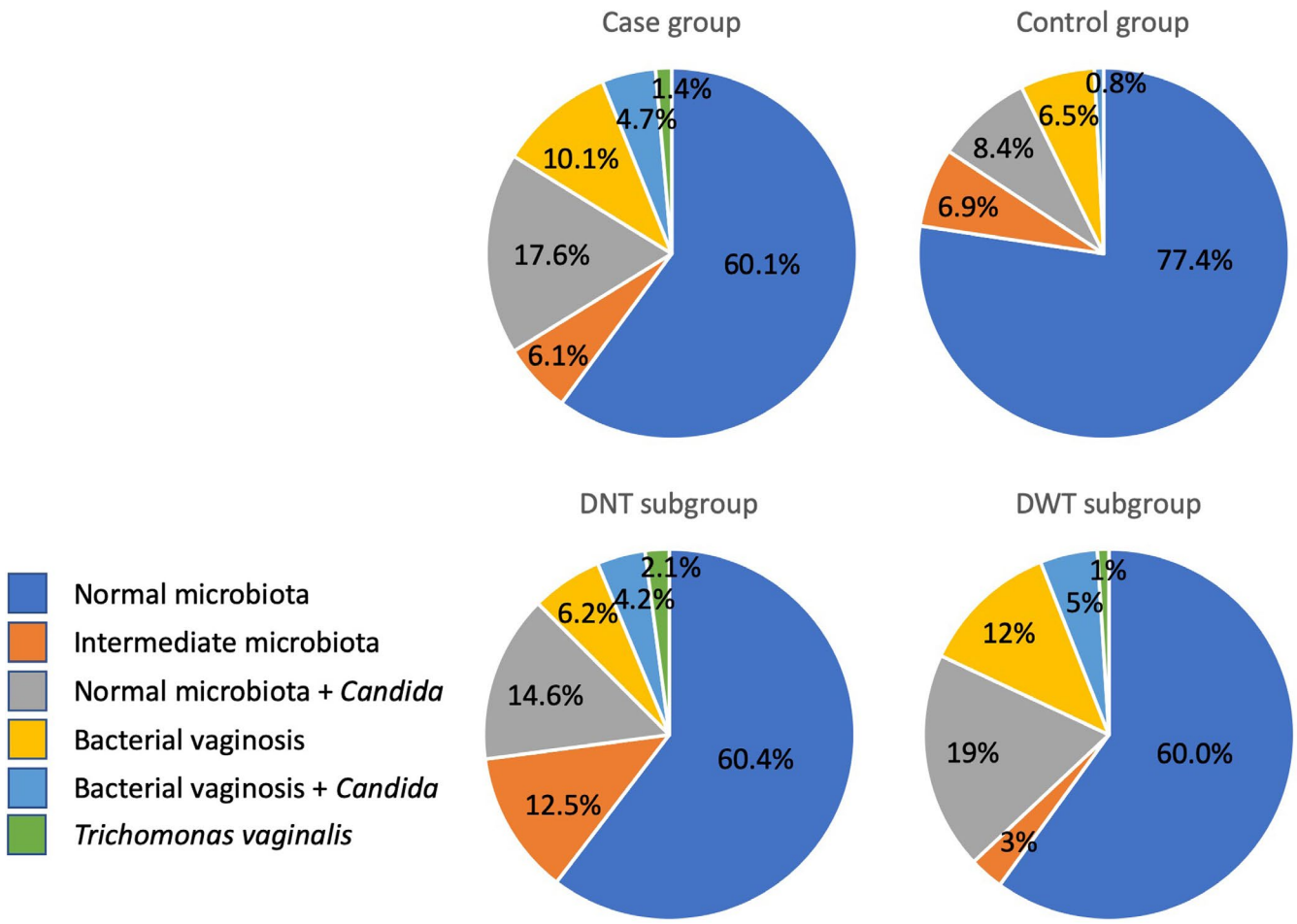

FIGURE 1 Vaginal microbiota among 539 cases and controls. (DWT, disease with treatment; DNT, disease without treatment)

TABLE 2 Vaginal microbiota among 539 cases and controls

Variable	Case group N (%)	Control group N (%)	Total N (%)	OR	95% CI	*p*-value
Participants	148 (27.5)	391 (72.5)	539 (100)			
Normal/intermediate microbiota[a]	98 (66.2)	330 (84.4)	428 (79.4)	0.36	0.23–0.56	<.001
Normal microbiota[a]	89 (60.1)	303(77.4)	394 (70.1)	0.45	0.30-0.67	<.001
Intermediate microbiota[a]	9 (6.1)	27 (6.9)	34 (6.3)	0.67	0.28-1.57	.36
Abnormal vaginal microbiota (any)[b]	50 (33.8)	61 (15.6)	111 (20.6)	2.76	1.78-4.27	<.001
Bacterial vaginosis (total)	22 (14.9)	28 (7.2)	50 (9.3)	2.26	1.25-4.1	.006
Bacterial vaginosis without *Candida*	15 (10.1)	25 (6.5)	40 (7.4)	1.65	0.85-3.23	.139
Bacterial vaginosis with *Candida*	7 (4.7)	3 (0.8)	10 (1.9)	6.4	1.64-24.17	.002
Candida colonisation (total)	33 (22.3)	36 (9.2)	69 (12.8)	2.83	1.69-4.75	<.001
Normal/intermediate microbiota with *Candida*	26 (17.6)	33 (8.4)	59 (10.9)	2.31	1.33-4.02	.002
Trichomoniasis [c]	2 (1.4)	0 (0.0)	2 (0.4)	-	-	.021

Abbreviations: OR, odds ratio; CI, confidence interval; N, number; N/A, not available.

[a]Without *Candida* colonisation or *Trichomonas vaginalis*.

[b]Including bacterial vaginosis, trichomoniasis and/or *Candida* colonization.

[c]Trichomoniasis was not documented in the control group, which did not allow calculation of odds for this parameter.

(DNT). Disease distribution and immunomodulatory therapy are shown in Table S1. The most frequent disease diagnosis was systemic lupus erythematosus (SLE), followed by Crohn's disease, ulcerative colitis, rheumatoid arthritis and Sjögren's syndrome. Maternal characteristics of the cases and controls are shown in Table 1.

3.2 | Vaginal microbiota

Evaluation of the routine antenatal screening smears showed that 50/148 (33.8%) women in the case group, and 61/391 (15.6%) women in the control group had an AVM. Out of the case group, we found that 13/48 (27.1%) women in the DNT subgroup, and 37/100 (37.0%) women in the DWT subgroup had an AVM, as shown in Figure 1.

The prevalence of pathogens among cases and controls is shown in Table 2. The chi-squared test showed a statistically significant difference in the occurrence of an AVM between cases and controls (33.8% vs 15.6%; $p < .001$). Prevalence of *Candida* spp. was higher among cases, compared with controls (22.3% vs 9.2%; $p < .001$). *Candida* colonisation with BV occurred significantly more often in the case group than in the control group (4.7% vs 0.8%; $p = .002$), as well as with normal or intermediate microbiota (17.6% vs 8.4%; $p = .002$). Significant differences were also found for BV (14.9% vs 7.2%; $p = .006$) and trichomoniasis (1.4% vs 0.0%; $p = .021$). Notably, BV without *Candida* did not differ significantly between the case and the control group (10.1% vs 6.4%; $p = .139$). Of note, trichomoniasis was not documented among the controls, which did not allow to calculate the odds ratio for this parameter.

While the rate of AVM was higher in the DWT subgroup, as compared to the DNT subgroup, this difference was not statistically significant (37.0% vs 27.1%, $p = .232$, Table 3). When comparing women in the DWT subgroup compared with those in the DNT subgroup, we observed a higher rate of BV and *Candida* colonisation among those of the DWT subgroup, which was, however, not statistically significant (17.0% vs 10.4%, $p = .292$ and 24.0% vs 18.8%, $p = .473$). Rates of trichomoniasis were higher in the DNT subgroup compared with the DWT subgroup, although the numbers were small (1.0% vs 2.1%; $p = .593$), as shown in Table 3.

3.3 | Obstetrical outcomes

Obstetrical outcomes are reported in Table S2. We found that 530/534 (99.3%) women experienced live birth, whereas 4/534 (0.7%) experienced stillbirth, whereof 2/148 (1.4%) occurred in the case group (one case in the DWT and in the DNT subgroup, each) and 2/386 (0.5%) in the control group. Data were missing for five infants in the control group.

Overall, the mean gestational age at delivery was 38.6 ± 2.6 weeks, with a mean of 38.7 ± 2.5 weeks in the case group and 38.5 ± 2.5 weeks in the controls. Women of the DWT subgroup delivered at a mean of 38.9 ± 2.7 weeks, compared to 38.4 ± 3.6 in the DNT subgroup. We found no significant difference with regard to the gestational age between cases and controls ($p = .416$), as well as between cases of the DWT vs the DNT subgroup ($p = .376$).

Overall, PTB was reported in 94/532 (17.7%) women, whereof 26/141 (18.4%) were cases, and 68/391 (17.4%) were controls (no data available for 7 cases; $p = .780$). The difference between the 9/45 (20.0%) cases with PTB in the DNT subgroup, and 17/96 (17.7%) in the DWT subgroup was not statistically significant ($p = .774$). We found no correlation between an AVM and PTB in our cohort, as shown in Table S3. Overall, the mean neonatal birthweight was 3216 ± 669 g, with a mean of 2961 ± 744 g in the case and

TABLE 3 Vaginal microbiota among 148 cases in the DWT and DNT subgroups

	DWT subgroup	DNT subgroup	Total			
Variable	***N* (%)**	***N* (%)**	***N* (%)**	**OR**	**95% CI**	***p*-value**
Participants	100 (67.6)	48 (32.4)	148 (100)			
Normal/intermediate microbiota[a]	63 (63.0)	35 (72.9)	98 (66.2)	0.63	0.29-1.35	.232
Normal microbiota[a]	60 (60.0)	29 (60.4)	89 (60.1)	0.98	0.49-1.99	.96
Intermediate microbiota[a]	3 (3.0)	6 (12.5)	9 (6.1)	0.21	0.05-0.91	.036
Abnormal vaginal microbiota (any)[b]	37 (37.0)	13 (27.1)	50 (33.8)	1.58	0.74-3.36	.232
Bacterial vaginosis (total)	17 (17.0)	5 (10.4)	22 (14.9)	1.76	0.61-5.09	.292
Bacterial vaginosis without *Candida*	12 (12.0)	3 (6.2)	15 (10.1)	2.05	0.55-7.62	.278
Bacterial vaginosis with *Candida*	5 (5.0)	2 (4.2)	7 (4.7)	1.21	0.23-6.48	.823
Candidosis (total)	24 (24.0)	9 (18.8)	33 (22.3)	1.37	0.58-3.23	.473
Normal/intermediate with *Candida*	19 (19.0)	7 (14.6)	26 (17.6)	1.37	0.53-3.53	.509
Trichomoniasis	1 (1.0)	1 (2.1)	2 (1.4)	0.46	0.03-7.76	.593

Abbreviations: OR, odds ratio; CI, confidence interval; DNT, disease without therapy; DWT, disease with therapy; N, number.

[a]Without *Candida* colonisation or *Trichomonas vaginalis*.

[b]Including bacterial vaginosis, trichomoniasis and/or *Candida* colonisation.

[c]Trichomoniasis was not documented in the control group, which did not allow calculation of odds for this parameter.

TABLE 4 Maternal and foetal complications among 148 cases in the DWT and DNT subgroups

Variable	DWT subgroup *N* (%)	DNT subgroup *N* (%)	*p*-value
Participants	100 (18.6)	48 (8.9)	
Maternal complications			
Pregnancy-induced hypertension	6 (6.1)	2 (4.3)	.654
Preeclampsia	2 (2.0)	2 (4.3)	.44
Gestational diabetes	9 (9.0)	4 (8.5)	.922
Foetal complications			
IUGR	2 (2.0)	1 (2.1)	.966
Death within first week of life	1 (1.0)	0 (0.0)	.496
NICU admission	13 (13.7)	8 (17.8)	.526
Foetal lung maturation	9 (9.4)	5 (11.1)	.748
Tocolysis	9 (9.4)	4 (8.9)	.926

Abbreviations: DNT, diasease without therapy; DWT, diasease with therapy; IUGR, intrauterine growth restriction; N, number; NICU, neonatal intensive care unit.

3308 ± 615 g in the control group, respectively ($p < .001$). Infants of the DWT and the DNT subgroup had a mean birthweight of 2949 ± 691 g and 2985 ± 851 g, respectively (no data available for 10 infants; $p = .796$).

Out of 532/539 (98.7%) cases and controls with available data, 255/532 (47.9%) of them resulted in vaginal delivery, 245/532 (46.1%) in caesarean section and 32/532 (6.0%) in instrumental delivery. The rate of vaginal delivery was significantly higher in the control group ($n = 199/391$), compared with the case group ($n = 56/148$), which was statistically significant (50.9% vs 39.7%; $p = .023$). Consequently, the rate of caesarean section was higher in the case group ($n = 75/148$), compared with the control group ($n = 170/391$), which was also statistically significant (53.2% vs 43.5%; $p = .047$).

We found no significant differences regarding maternal and foetal complications among the cases with and without immunomodulatory therapy during pregnancy (Table 4).

4 | DISCUSSION

Autoimmune diseases and immunomodulatory therapies both alter the balance among the key players in our immune system, and therefore, potentially contribute to vaginal dysbiosis and infection. Pregnant women with inflammatory autoimmune diseases have an a priori increased risk for PTB, and it is, therefore, important to define, prevent and modify further risk factors for this multifactorial event. To the best of our knowledge, our study is the first evaluating the vaginal microbiota of pregnant women with inflammatory rheumatic and inflammatory bowel diseases. We found an increased risk for an AVM in women with inflammatory rheumatic or inflammatory bowel diseases compared with healthy pregnant controls. Predominantly, the rates of *Candida* colonisation, but also of BV and trichomoniasis, were significantly higher in women with inflammatory diseases, which certainly suggest a potential influence of inflammatory diseases on the vaginal microbiota. The increased risk of women with autoimmune conditions for an AVM might theoretically be attributed to an inappropriate immune response on the basis of the pathogenic mechanism of autoimmunity itself.[17] Although we observed a trend towards an increased risk of AVM in women who received immunosuppressive treatment, compared with those who did not, this finding was not statistically significant.

In our study, vaginal colonisation with *Candida* spp. was increased among pregnant women with inflammatory autoimmune disease. Previously published studies of healthy pregnant women reported *Candida* colonisation rates of 11%–14% at early gestation,[7,18,19] which are comparable to our findings in the control group. With regard to the prevalence of *Candida* among healthy pregnant women, significantly higher rates have been reported in a study from Papua New Guinea, which might reflect the importance of genetic factors influencing susceptibility for infections.[20] Indeed, it is well known that *Candida* occurs more frequently in patients under immunosuppression.[21] In animal models, prednisolone and cyclophosphamide were the most potent factors for high fungal burden and long-lasting infections in association with immunosuppression.[10,21] To the contrary, Aikawa et al found a very low prevalence of *Candida* in patients with rheumatic diseases who received anti-TNF-therapy.[14] In their study, only one out of 194 patients (0.5%) had *Candida*, and none of them experienced systemic candidosis.[14] Interestingly, the authors only evaluated cases with symptomatic infection among women with autoimmune disorders, while all women in our study were asymptomatic at the time of screening.

Our findings support the hypothesis that inflammatory autoimmune disease in pregnant women might influence local immune mechanisms and thus increase the individual susceptibility for fungal infections. The role of the adaptive immunity in *Candida*-specific defence mechanisms was investigated in several immune-compromised pathologies, such as HIV infection, previous organ transplantation, glucocorticoid therapy or antineoplastic chemotherapy. In this immunocompromised group of patients, the decreased T-cell-mediated immune response seems to be associated

with increased susceptibility to vulvovaginal candidosis.[22] However, this theory needs to be further investigated.

In contrast to fungal infections, where the risk of PTB is yet under debate, BV is clearly known to be a risk factor for this multifactorial event.[7,19,23,24] *Gardnerella vaginalis* is the predominant microbe in women with BV and is, therefore, of particular interest. In 2014, Schilling et al.[13] described an urogenital *G. vaginalis* biofilm in women with inflammatory bowel disease. The urine of these women was frequently colonised with *Gardnerella*, and women who received steroid therapy were more often colonised. The authors attributed their findings to an epithelial barrier dysfunction of the genital tract.[13] We were unable to compare our findings with those of their study, as we did not analyse urine samples. Donmez et al.[25] also linked BV to autoimmune antibody positivity. In their study, women with antibody positivity showed BV rates of 13.8%, compared to 2.4% in the antibody-negative group. Still, it is not clear whether BV induces autoantibody production or whether autoimmunity increases the risk of BV. Based on our findings, we cannot confirm either of these hypotheses, as we did not find significant differences in BV rates among our case groups. However, the BV rates that we found in our cohort were comparable to those of previously published studies. The risk of infections in patients on immunotherapy is well known. Unfortunately, the scarcity of data on vaginal infections related to immunotherapy in inflammatory rheumatic or bowel diseases in pregnancy does not allow extensive comparisons. Further studies are needed to evaluate whether DMARDs or steroid therapy pose an additional risk for vaginal infections.

The primary reasons why we aimed to identify whether or not women with inflammatory rheumatic diseases are at risk of AVM was to avoid a potential accumulation of additional risk factors for PTB among high-risk pregnant women. With regard to PTB, we found no significant differences between cases and controls, as well as between cases with and without an immunosuppressive therapy. Notably, the PTB rate among our cohort is significantly higher than the overall PTB rate in the country, as we only observed high-risk women at our tertiary referral centre. Austrian perinatal registry data suggest an average PTB rate that ranges from 7.3% in 2018 [26] to 8.7% in 2014 [27] and 11.1% in 2008.[28] Dominitz et al.[29] described a lower PTB rate than we found in our study, along with the findings of Smith et al,[30] who described PTB rates of 10.9% in women with ankylosing spondylitis, and of 13.7% in those with psoriatic arthritis; all of which are considerably lower than in our study. In women with SLE who are known to experience more complicated pregnancies than women with other autoimmune disorders, reported PTB rates are considerably higher, ranging from 20% to 54%.[3,31,32] We suggest that the high PTB rate in our study is likely due to the tertiary study setting. Overall, the PTB rate of 18.4% among women in our case group is still considered to be satisfactory and comparable with the available literature.[27]

The lower mean birthweight that we found among the case group, could theoretically be attributed to impaired placental function in women with inflammatory rheumatic diseases. It is well known that disease activity is an important risk factor for adverse obstetric outcomes, including PTB and intrauterine growth restriction (IUGR).[1,3,31-33] Unfortunately, we did not routinely record disease activity in our study, and this prevents to further analyse this point. Our findings are in line with available literature, which suggests that inflammatory rheumatic or bowel disease might lead to IUGR through endothelial damage from circulating antiangiogenic factors and other inflammatory molecules in combination with pre-existing maternal vascular risk factors.[34]

The caesarean section rate that we found was significantly higher among women with autoimmune conditions (Table S2). Increased rates of caesarean section have already been reported for women with autoimmune diseases.[1,4,30,35] Chakravarty et al[36] reported that 39.4% of pregnant women suffering from SLE, and 37.2% with rheumatoid arthritis required a caesarean section. The recently published study by Smith et al[30] reported a caesarean section rate of 33.3% in women with ankylosing spondylitis and 48.7% in women with psoriatic arthritis. Again, we assume that the reason for the high caesarean section rate that we observed was attributed to the fact that we solely care for high-risk pregnancies at our centre. Moreover, the decision-making regarding the mode of delivery is often compromised from fear of complications in late pregnancy or peripartum, which poses a bias to clinical decision-making towards the operative mode of delivery. In addition, the higher caesarean section rate among the case group might also be attributed to the increased rate of primiparous women in this group, who are known to have an increased likelihood for caesarean section, compared with multiparous women.[37,38]

We are aware that our study has several limitations, above all due to its retrospective character. First and foremost is the fact that inflammatory rheumatic and inflammatory bowel diseases are rare conditions; although we analysed our in-house data for a long time period, the number of eligible cases remained relatively small. Moreover, there are several risk factors that might have been associated with vaginal infections (eg stress, pre-existing comorbidities, antibiotic or probiotic treatment) that were not considered, since these data were not part of our database. As it is known that complication rates vary between different inflammatory disorders, with the highest complication rates among women with SLE, it is difficult to compare our results to that of previously published studies, as most of them referred to particular diseases. Finally, we were unable to report maternal comorbidities and disease activity during pregnancy, even though these factors are known to increase the risk for adverse obstetrical outcomes.

5 | CONCLUSIONS

Our findings indicate that pregnant women with inflammatory rheumatic and inflammatory bowel diseases are at increased risk for an abnormal vaginal microbiota, including BV and the colonisation with *Candida* species. Considering the risk that autoimmune conditions pose on obstetrical outcomes and complications that may arise from consecutive vaginal dysbiosis and infection, we suggest that affected

women need to be consequently screened and treated during pregnancy. Further prospective studies are needed to confirm our findings, as well as to evaluate whether there is an influence of autoimmune diseases and immunosuppressive treatments on the vaginal microbiota.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Klara Rosta: Data curation (lead); Investigation (lead); Validation (equal); Writing-original draft (lead). **Antonia Mazzucato-Puchner:** Data curation (equal); Investigation (equal); Project administration (equal); Resources (supporting); Writing-review & editing (equal). **Herbert Kiss :** Resources (equal); Supervision (equal); Writing-review & editing (equal). **Veronika Malik:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Writing-review & editing (supporting). **Peter Mandl:** Formal analysis (supporting); Investigation (equal); Methodology (supporting); Supervision (supporting); Validation (supporting); Writing-original draft (supporting); Writing-review & editing (supporting). **Ljubomir Petricevic:** Conceptualization (supporting); Supervision (equal); Validation (equal); Writing-review & editing (supporting). **Philipp Foessleitner:** Data curation (supporting); Resources (supporting); Writing-review & editing (supporting). **Inbal Shafran:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Software (supporting); Writing-review & editing (supporting). **Wilhelm Temsch:** Formal analysis (lead); Software (lead); Validation (lead). **Alex Farr:** Conceptualization (lead); Investigation (lead); Methodology (lead); Project administration (lead); Supervision (lead); Writing-original draft (supporting); Writing-review & editing (lead).

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REFERENCES

1. Huang VW, Habal FM. From conception to delivery: managing the pregnant inflammatory bowel disease patient. *World J Gastroenterol*. 2014;20(13):3495-3506.
2. Cortés-Hernández J, Ordi-Ros J, Paredes F, Casellas M, Castillo F, Vilardell-Tarres M. Clinical predictors of fetal and maternal outcome in systemic lupus erythematosus: a prospective study of 103 pregnancies. *Rheumatology (Oxford)*. 2002;41(6):643-650.
3. Marder W, Littlejohn EA, Somers EC. Pregnancy and autoimmune connective tissue diseases. *Best Pract Res Clin Rheumatol*. 2016;30(1):63-80.
4. Chen JS, Ford JB, Roberts CL, Simpson JM, March LM. Pregnancy outcomes in women with juvenile idiopathic arthritis: a population-based study. *Rheumatology (Oxford)*. 2013;52(6):1119-1125.
5. Furer V, Rondaan C, Heijstek M, et al. Incidence and prevalence of vaccine preventable infections in adult patients with autoimmune inflammatory rheumatic diseases (AIIRD): a systemic literature review informing the 2019 update of the EULAR recommendations for vaccination in adult patients with AIIRD. *RMD Open*. 2019;5(2):e001041.
6. Romero R, Hassan SS, Gajer P, et al. The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term. *Microbiome*. 2014;2:18.
7. Farr A, Kiss H, Hagmann M, Marschalek J, Husslein P, Petricevic L. Routine use of an antenatal infection screen-and-treat program to prevent preterm birth: long-term experience at a tertiary referral center. *Birth*. 2015;42(2):173-180.
8. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371(9606):75-84.
9. Kemp MW. Preterm birth, intrauterine infection, and fetal inflammation. *Front Immunol*. 2014;5:54.
10. Wang L, Wang C, Mei H, et al. Combination of estrogen and immunosuppressive agents to establish a mouse model of candidiasis with concurrent oral and vaginal mucosal infection. *Mycopathologia*. 2016;181(1–2):29-39.
11. Méndez-Martínez S, García-Carrasco M, Cedillo-Ramírez ML, et al. Genital mycoplasma infection among Mexican women with systemic lupus erythematosus. *Int J Gynaecol Obstet*. 2017;138(1):17-22.
12. Machado AA, Zorzi AR, Gléria AE, Donadi EA. Frequency of Mycoplasma hominis and Ureaplasma urealyticum infections in women with systemic lupus erythematosus. *Rev Soc Bras Med Trop*. 2001;34(3):243-247.
13. Schilling J, Loening-Baucke V, Dörffel Y. Increased Gardnerella vaginalis urogenital biofilm in inflammatory bowel disease. *J Crohns Colitis*. 2014;8(6):543-549.
14. Aikawa NE, Rosa DT, Del Negro GM, et al. Systemic and localized infection by Candida species in patients with rheumatic diseases receiving anti-TNF therapy. *Rev Bras Reumatol Engl Ed*. 2016;56(6):478-482.
15. Hillier SL, Krohn MA, Nugent RP, Gibbs RS. Characteristics of three vaginal flora patterns assessed Gram stain among pregnant women. *Am J Obstet Gynecol*. 1992;166(3):938-944.
16. Nürnberger L. *Die Erkrankungen der Scheide*. München, Germany: J.F. Bergmann; 1930:800.
17. Maddur MS, Vani J, Lacroix-Desmazes S, Kaveri S, Bayry J. Autoimmunity as a predisposition for infectious diseases. *PLoS Pathog*. 2010;6(11):e1001077.
18. Farr A, Petricevic L. Infektionsscreening in der Schwangerschaft zur Reduktion der Frühgeburtenrate: Langzeitanalyse und neue Erkenntnisse. *Spekulum*. 2015;3:6-9.
19. Farr A, Kiss H, Holzer I, Husslein P, Hagmann M, Petricevic L. Effect of asymptomatic vaginal colonization with Candida albicans on pregnancy outcome. *Acta Obstet Gynecol Scand*. 2015;94(9):989-996.
20. Klufio CA, Amoa AB, Delamare O, Hombhanje M, Kariwiga G, Igo J. Prevalence of vaginal infections with bacterial vaginosis, Trichomonas vaginalis and Candida albicans among pregnant women at the Port Moresby General Hospital Antenatal Clinic. *P N G Med J*. 1995;38(3):163-171.
21. Foldvari M, Radhi J, Yang G, He Z, Rennie R, Wearley L. Acute vaginal candidosis model in the immunocompromized rat to evaluate delivery systems for antimycotics. *Mycoses*. 2000;43(11–12):393-401.

22. Sustr V, Foessleitner P, Kiss H, Farr A. Vulvovaginal candidosis: current concepts, challenges and perspectives. *J Fungi*. 2020;6:267.
23. Kiss H, Petricevic L, Husslein P. Prospective randomised controlled. trial of an infection screening programme to reduce the rate of preterm delivery. *BMJ*. 2004;329(7462):371.
24. Leitich H, Kiss H. Asymptomatic bacterial vaginosis and intermediate fl ora as risk factors for ad-verse pregnancy outcome. *Best Pract Res Clin Obstet Gynaecol*. 2007;21:375-390.
25. Donmez HG, Cagan M, Fadiloglu E, Unal C, Onder SC, Beksac MS. Is bacterial vaginosis associated with autoimmune antibody positivity? *Cytopathology*. 2020;31(4):298-302.
26. Medical and sociomedical characteristics; 2020. http://www.statistik.at/web_en/statistics/PeopleSociety/population/births/medical_and_sociomedical_characteristics/index.html. Accessed Jul 16, 2020.
27. Chawanpaiboon S, Vogel JP, Moller AB, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health*. 2019;7(1):e37-e46.
28. Murphy M, McLoughlin G. Born too soon: preterm birth in Europe trends, causes and prevention. *Entre Nous*. 2015;81:10-12.
29. Dominitz J, Young J, Boyko E. Outcomes of infants born to mothers with inflammatory bowel disease: a population-based cohort study. *Am J Gastroenterol*. 2002;97(3):641-648.
30. Smith CJF, Bandoli G, Kavanaugh A, Chambers CD. Birth outcomes and disease activity during pregnancy in a prospective cohort of women with psoriatic arthritis and ankylosing spondylitis. *Arthritis Care Res (Hoboken)*. 2020;72(7):1029-1037.
31. Clowse MEB. Lupus activity in pregnancy. *Rheum Dis Clin North Am*. 2007;33(2):237-252.
32. Simard JF, Chaichian Y, Rossides M, Wikstrom A-K, Shaw GM, Druzin ML. Preterm delivery phenotypes in systemic lupus erythematosus pregnancies. *Am J Perinatol*. 2019;36(9):964-968.
33. Jakobsson GL, Stephansson O, Askling J, Jacobsson LTH. Pregnancy outcomes in patients with ankylosing spondylitis: a nationwide register study. *Ann Rheum Dis*. 2016;75(10):1838-1842.
34. Soh MC, Nelson-Piercy C. Biomarkers for adverse pregnancy outcomes in rheumatic diseases. *Rheum Dis Clin North Am*. 2017;43(2):201-14.
35. Remaeus K, Johansson K, Askling J, Stephansson O. Juvenile onset arthritis and pregnancy outcome: a population-based cohort study. *Ann Rheum Dis*. 2017;76(11):1809-1814.
36. Chakravarty EF, Nelson L, Krishnan E. Obstetric hospitalizations in the United States for women with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum*. 2006;54(3):899-907.
37. Patel RR, Peters TJ, Murphy DJ; ALSPAC Study Team. Prenatal risk factors for Caesarean section. Analyses of the ALSPAC cohort of 12,944 women in England. *Int J Epidemiol*. 2005;34(2):353-367.
38. Abu-Heija A, Rasheed R, el-Qaraan O. Effect of age and parity on primary caesarean section rates. *Clin Exp Obstet Gynecol*. 1998;25(1–2):38-39.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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