EXAMINING THE IMPACT OF THE UTERINE MICROBIOME ON REPRODUCTIVE OUTCOMES

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Background: Although the clinical significance of the uterine microbiome remains relatively poorly understood, it has been suggested that an imbalance of microorganisms within the reproductive tract can have an impact on reproductive outcomes.[1-3] Specifically, this imbalance has been linked to a condition known as chronic endometritis, which has been associated with both recurrent implantation failure and pregnancy loss.[4-5] In recent years, assessments such as the Endometrial Microbiome Metagenomic Analysis/Analysis of Infectious Chronic Endometritis (EMMA/ALICE) tests have been developed to determine 1) the extent of naturally-occurring bacteria, particularly *Lactobacilli*, that promote implantation and pregnancy as well as 2) the presence of pathogenic bacteria within the uterine microbiome that can impede favorable outcomes.[6-7] However, data examining the effect that treatment of a microbiological disequilibrium of the endometrium can have on reproductive outcomes are limited.

Objective: The purpose of this study was to compare the reproductive outcomes of patients following an endometrial biopsy and assessment of the uterine microbiome.

Materials and Methods: All single, autologous, euploid frozen embryo transfers succeeding an endometrial biopsy with EMMA/ALICE screening from January 1, 2021 to December 31, 2023 were included. Cycles were stratified based on whether patients had a negative test, or a positive result (deficient *Lactobacilli* and/or positive pathogenic bacteria) with subsequent intervention (probiotics and/or antibiotics). Patient demographics and cycle data were collected. The primary outcome was the live birth rate per embryo transfer while secondary outcomes included positive beta HCG, clinical pregnancy, and miscarriage rates. To adjust for possible confounders including age, body mass index, anti-Mullerian hormone level, gravidity, parity, cycle protocol, and a history of prior embryo transfers as well as account for multiple cycles completed per patient, generalized estimating equation models were used to calculate risk ratios and 95% confidence intervals.

Result(s): A total of 66 and 90 single, autologous, euploid frozen embryo transfer cycles following a negative and positive endometrial biopsy, respectively, were included. Demographics and cycle characteristics were generally similar between the two groups as exemplified in Table 1. LBRs were also similar between the two groups, with 27 (40.91%) versus 36 (40.00%) live births recorded after an endometrial biopsy with a negative and positive screen, respectively. In the adjusted models depicted in Table 2, the risk of live birth between the two groups was not statistically different (RR=1.01, 95% CI: 0.51-1.97). Similar to live birth rates, there was no difference in positive beta HCG, miscarriage, or clinical pregnancy rates between those with a negative or positive EMMA/ALICE screen in the adjusted models.

Conclusion(s): Reproductive outcomes following treatment of uterine microbiome abnormalities were similar to patients with no initial evidence of microbiological imbalance. While this may support the use of endometrial testing for naturally-occurring and pathogenic bacteria when faced with recurrent implantation failure or pregnancy loss, these results should be interpreted with caution given that 1) no test of cure was completed to confirm resolution, and 2) outcomes cannot be predicted if patients with a positive screen did not undergo treatment. Consequently, further investigation should be completed to understand the role of intervention in improving reproductive outcomes.

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Demographics and Cycle Characteristics	Cycles following negative screen N=66	Cycles following positive screen N=90
Number of previous frozen embryo transfer cycles	1.71	2.27
Number of previous euploid frozen embryo transfer cycles	1.44	1.63
Age (years) at the time of biopsy	35.92	36.23
Anti-Müllerian hormone (ng/mL) at the time of biopsy	2.84	3.59
Body mass index (kg/m^2) at the time of biopsy	26.76	26.69
Gravidity at the time of biopsy	1.09	1.47
Parity at the time of biopsy	0.20	0.26
Frozen embryo transfer cycle preparation		
Programmed/medicated	43	59
Natural/modified natural	23	31
Peak Estradiol (pg/mL)	320.97	406.76
Dominant follicle size (mm)	20.40	20.50
Endometrial thickness (mm)	8.72	8.70

Table 1. Patient demographics and cycle characteristics

Outcome	N (%)	Unadjusted	Adjusted*
Positive beta HCG			
Cycles following negative screen	41 (62.12%)	Ref	Ref
Cycles following positive screen	56 (62.22%)	1.05 (0.54, 2.03)	1.03 (0.51, 2.05)
Clinical pregnancy			
Cycles following negative screen	32 (48.48%)	Ref	Ref
Cycles following positive screen	45 (50.00%)	1.25 (0.66, 2.34)	1.30 (0.67, 2.5)
Miscarriage			
Cycles following negative screen	5 (7.58%)	Ref	Ref
Cycles following positive screen	9 (10.00%)	1.41 (0.40, 4.89)	1.35 (0.40, 4.62)
Live birth			
Cycles following negative screen	27 (40.91%)	Ref	Ref
Cycles following positive screen	36 (40.00%)	0.97 (0.50, 1.87)	1.01 (0.51, 1.97)

Table 2. Univariable and multivariable generalized estimating equations analyses comparing reproductive outcomes

*Adjusted for age, BMI, AMH, gravidity, parity, cycle protocol, and a history of previous embryo transfers

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