Stress, Menstruation, and Contraceptive Use in Young Adults: Insights from a Microbiome Study

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INTRODUCTION

The human microbiome, composed of bacteria, fungi, viruses, archaea, protozoa, and their metabolite products, plays a critical role in human health and disease (1). The gut microbiome, in particular, has been linked to a variety of conditions, including cancer, obesity, and neurological disorders (2,3,4,5). Stress and lifestyle choices—such as diet, exercise, and sleep—are known to influence the composition of the gut microbiome and the metabolites it produces. However, less is known about the relationship of these factors with the vaginal microbiome. While the vaginal microbiome plays a key role in maintaining vaginal health, imbalances in its composition have also been linked to diseases such as endometrial and ovarian cancer (6). Prior work from our group has shown that the vaginal microbiota is influenced by factors such as the menstrual cycle, birth control, and diet (7). This study builds upon past work by exploring how stress and lifestyle choices relate to the gut and vaginal microbiomes, focusing on microbial composition and metabolite production in a young, healthy population. We present initial findings on the associations between stress and menstruation over time in young adults.

OBJECTIVES

The present research study seeks to:

1) Examine whether menstruation status is a significant predictor of stress levels in a cohort of young adults.
2) Analyze whether different types of birth control affect stress levels, both independently and in interaction with menstruation status.
3) Explore how stress scores fluctuate over time and whether these trends differ based on menstruation and birth control status.

METHODS

This longitudinal dataset was collected at Wellesley College in Fall 2022. Students aged 18+ with a vagina (n=63) participated in a 10-week study on the effects of mood and lifestyle factors on the gut and vaginal microbiomes. Each participant completed at least one Depression Anxiety and Stress Scales (DASS) survey.

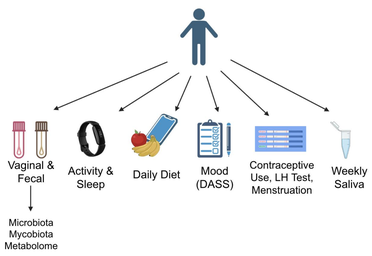

Figure 1. Samples and data provided by participants. Two vaginal and two fecal samples were provided daily by each participant for 10 weeks. Daily diet and nutritional information were collected by an app developed by the Wellesley IT Department, and activity and sleep data were collected by FitBit. Mood was assessed once a week by a DASS survey. Saliva was collected weekly on the same day as mood assessment (Table 1).

Stress Score	0-7	8-9	10-12	13-16	17- 40
Stress Category	Normal	Mild	Moderate	Severe	Extremely Severe

Table 1. A higher stress score indicates a higher stress level on the stress subscale of the Depression Anxiety and Stress Scales (DASS) survey.

	No Menstruate	Menstruate	Total
No Birth Control	0	32	32
Systemic Combined (E&P)	2	14	16
Local P	6	2	8
Systemic P only	4	3	7
Total	12	51	63

Table 2. Menstruation status and type of birth control for the participants (E = estrogens; P = progestins).

10% of all mood surveys had approximately one missing response (out of 21 questions). Under the assumption that this low-frequency missingness occurred completely at random, missing responses were imputed by randomly sampling from a subject's answered questions within the same category (*e.g.*, stress). A higher stress score indicates a more severe stress level (Table 1).

Mixed-effects models were used to analyze the stress levels of participants over time. Polynomial terms were included to model non-linear patterns of stress level over time. ANOVA was used to test whether birth control or menstruation status significantly improved a model based on time alone.

Birth control was categorized into four types: 1) no birth control, 2) systemic combined (estrogens & progestins), 3) local progestins, and 4) systemic progestins only. Participants were classified as either menstruating or non-menstruating (Table 2), based on whether they menstruate at all, not at a specific time. All participants were either menstruating, on birth control, or both.

RESULTS

Using ANOVA, we found that birth control did not significantly enhance the model's ability to predict stress (p = 0.30). However, predicting stress based on menstruation status rather than birth control led to a significant improvement in model fit (p = 0.05), indicating that menstruation status is a better indicator of stress levels than birth control use.

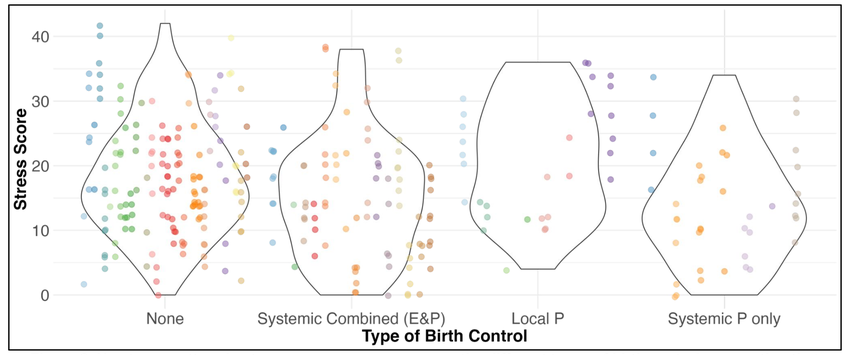

Figure 2. No strong pattern in stress scores emerges across the different types of birth control, suggesting that birth control alone is not a strong predictor of stress scores in this dataset. Most of the participants are in either the "no birth control" or "systemic combined (estrogens & progestins)" categories, indicated by a larger number of unique colors representing different study IDs within each category.

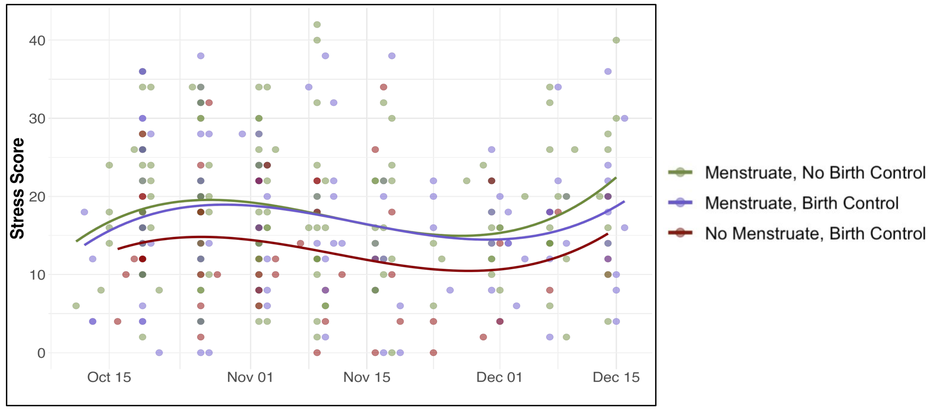

Figure 3. Across time, participants who menstruated, regardless of their birth control status, were significantly more stressed than participants who did not menstruate (p=0.05).

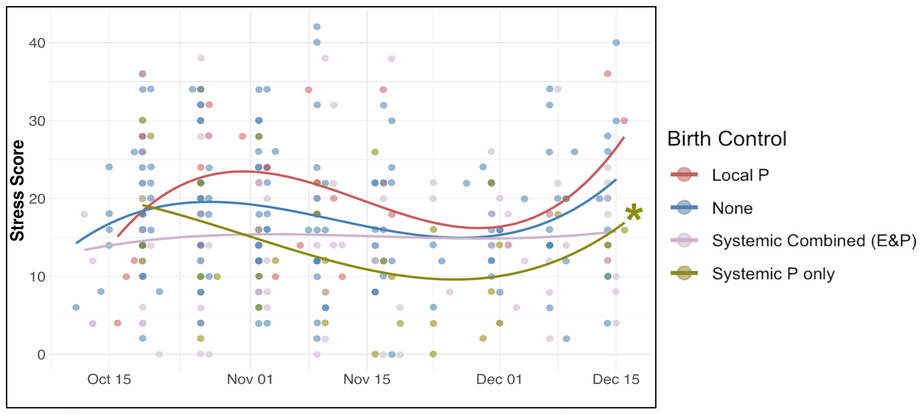

Figure 4. Participants using systemic progestin-only birth control exhibited trends in stress scores that were significantly different over time compared to those using no birth control (ANOVA, *p ≈ 0.01). The trend lines suggest a consistently lower stress score in the systemic progestin-only group.

CONCLUSIONS & LIMITATIONS

Conclusions

- Participants who menstruated had higher stress scores than participants who did not menstruate, regardless of the type of birth control used. Findings suggest that menstruation status is more strongly associated with stress levels than birth control type among participants in the present study.
- Participants using systemic progestin-only birth control showed significantly different stress score trends compared to those not using birth control (p = 0.01). Trend lines indicate consistently lower stress scores in the systemic progestin-only group.

Limitations

- The number of mood surveys completed by each subject varied from 1-9 submitted surveys. As a result, there are missing data where we cannot assume that the missingness is completely at random.
- Our sample size limits the statistical power of our analyses. Since there are no study participants who are both not on birth control and not menstruating, the results cannot be generalized to individuals outside the study who fall into this category.

DISCUSSION

- The gut microbiome has been implicated in stress and anxiety via the gut-brain axis. Investigating the interaction between stress and the gut and vaginal microbiomes is critical in understanding possible mechanisms of how life experiences and environmental factors impact these important microbiomes.
- We are currently analyzing gut and vaginal microbiota, as well as fungal (mycobiota) sequence data, with plans to incorporate metabolomics to explore the relationship between stress, lifestyle choices, and key aspects of these microbiomes. This high-resolution, longitudinal analysis will provide insights into the possible crosstalk between the gut and vaginal microbiomes and their impact on women's health and disease (8). In the future, we hope to identify early indicators of gynecologic or obstetric risk in the long term.

REFERENCES

(1) Ursell, L.K., *et al.* Defining the human microbiome. Nutrition reviews 70.suppl_1 (2012): S38-S44.
(2) Morais, L. H., *et al.*. The gut microbiota–brain axis in behaviour and brain disorders. Nature Reviews Microbiology 19.4 (2021): 241-255.
(3) Shreiner, A.B., *et al.*, The gut microbiome in health and in disease." Current opinion in gastroenterology 31.1 (2015): 69.
(4) Mawe, G. M., and J.M. Hoffman. Serotonin signalling in the gut—functions, dysfunctions and therapeutic targets. Nature reviews Gastroenterology & Hepatology 10.8 (2013): 473-486.
(5) Tetel, M.J., de Vries, G.J., Melcangi, R.C., Panzica, G., & O'Mahony, S.M. Steroids, stress and the gut microbiome-brain axis. *Journal of Neuroendocrinology*, 30(2), (2018): e12548. doi: 10.1111/jne.12548. PMID: 29024170; PMCID: PMC6314837.
(6) Walther-António, M. R., *et al.* Potential contribution of the uterine microbiome in the development of endometrial cancer. Genome medicine, 8(1), (2016): 122.
(7) Song, S. D., *et al.* "Daily vaginal microbiota fluctuations associated with natural hormonal cycle, contraceptives, diet, and exercise. MSphere 5.4 (2020): e00593-20.
(8) Graham, M.E., Herbert, W.G., Song, S.D., Raman, H.N., Zhu, J.E., Gonzalez, P.E., Walther-António, M.R.S., & Tetel, M.J. Gut and vaginal microbiomes on steroids: implications for women's health. *Frontiers in Neuroendocrinology*, *50*, (2023): 100830. doi: 10.1016/j.yfrne.2023.100830.

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