Team BELI: The Relationship Between Gut Motility and the Phases of the Menstrual Cycle

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Abstract

Female reproductive hormones fluctuate significantly throughout the menstrual cycle, influencing gastrointestinal motility and function. These changes in gut motility contribute to gastrointestinal symptoms such as flatulence, diarrhea, and constipation. When transit time is faster, fewer carbohydrates are absorbed in the small intestine, leading to increased microbial **fermentation** and gas production in the large intestine. Given the established effects of reproductive hormones on gut motility, we hypothesized that we would detect increased gut microbial gas production during menstruation, when estradiol and progesterone are lowest and transit time is fastest. However, due to a lack of tools to measure gut microbial gas production longitudinally, the literature lacks a formal test of this hypothesis. Therefore, we used a novel tool, the Smart Underwear device, to measure gut microbial gas production as a proxy for intestinal transit time. Participants wore the device for a total of nine days over the course of one menstrual cycle. On device-wearing days, participants logged the meals they consumed. Additionally, participants used Luteinizing Hormone test strips in the middle of their cycle, approximately on day 14, to confirm ovulation timing and more precisely map menstrual cycle phases. We extracted and analyzed the data from our returned Smart Underwear devices to measure gut microbial gas production as an indicator of intestinal transit time at different phases of the menstrual cycle, providing the first longitudinal measurements of gut microbial gas production across the menstrual cycle. Initial findings suggest that microbiome activity varies significantly across phases, with heightened activity during ovulation. Our analysis revealed that higher concentrations of flatulence peaks occurred during the ovulation phase.

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I. Introduction to the Problem

The field of female health is one that is particularly under researched. Currently, females remain underrepresented across clinical trials, reducing the generalizability of findings from these trials while reinforcing systemic biases (Kwiatkowski et al., 2013). This creates a knowledge gap in the functional differences between males and females, especially as it pertains to menstrual and reproductive health in females. According to a 2018 study, females surveyed reported more severe and frequent symptoms of Irritable Bowel Syndrome (IBS), with a rate of four times greater than males (Kim, 2018). Oftentimes, females with gut and digestive issues are misdiagnosed or do not even receive a diagnosis. In the Kim study, there is evidence that males and females have different treatment efficiencies and treatment for females are limited although many gastrointestinal diseases are more common amongst females. Similarly, there are no biological differences between the dissected gastrointestinal tracts of males and females, yet females are impacted more by disorders and diseases of the gut (Pigrau et al., 2015). Both menstrual and gastrointestinal pain are often self-reported and diagnosed because there are few efficient quantitative ways to measure them (Negriff, 2015). Since menstrual and gastrointestinal pain categories present themselves similarly, there is a high occurrence of misdiagnosis or delayed diagnosis of gastrointestinal diseases in females (Seear, 2009). Finally, empirical research has recorded observable changes in bowel and gut activity during the menstrual cycle, with females also reporting more experiences with GI symptoms than males (Van Den Houte et al., 2018). Thus, further research must be conducted to explain why females experience more gastrointestinal symptoms while on their menstrual cycles.

The current disparities that permeate the field of female health research can be explained by various social determinants of health. Variation in health and research opportunities across

social groups is not caused by innate biological differences, but instead by the social meaning behind group differences that are imbued with hierarchy and power. Likewise, females face higher rates of chronic conditions, pain disorders, and autoimmune disease than males, yet these issues are understudied and misdiagnosed in medicine due to gender bias (Short & Zacher, 2022). Historically omitting females from the early stages of clinical trials has led to a lack of data on how diseases impact female health. Since illnesses can manifest differently in females versus males, it is essential to have data on both sexes (Bender, 2017). Thus, research directed towards female health issues will help to close the knowledge gaps born out of health disparities.

This study aimed to contribute literature to the field of female health research, especially in the areas of gastrointestinal health and menstrual health. The disproportionate rate of IBS misdiagnosis amongst females identifies an important research focus, as the occurrence of gastrointestinal symptoms during the menstrual cycle is poorly understood. Working to understand the changes that occur in the gut throughout the menstrual cycle will establish a clearer correlation between gastrointestinal symptoms and menstrual symptoms, therefore reducing the misdiagnosis of female patients with abnormal gut function and reducing health disparities.

II. Menstrual Cycle

A. Female Health and the Menstrual Cycle

The female reproductive system undergoes regular cycle changes known as the menstrual cycle, preparing the body for ovulation and potential pregnancy. Normal menstrual frequency is defined as cycles occurring every twenty-one to thirty-five days starting when females reach

puberty (median age of 12.4) and ceasing at the onset of **menopause** (average onset age 51) (Thiyagarajan et al., 2025). The onset of menstruation is caused by the rise and fall of multiple hormones most notably luteinizing hormone (LH) and follicle stimulating hormone (FSH) which regulate estrogen and progesterone production (Reed & Carr, 2000; Thiyagarajan et al., 2025).

The menstrual cycle encompasses the ovarian cycle and the uterine cycle which fluctuate in conjunction with one another to regulate egg release, endometrial preparation, and hormone secretion (Reed & Carr, 2000). The ovarian cycle describes the changes occurring within the ovary, specifically the development and release of an egg, while the uterine cycle focuses on the changes in the lining of the uterus, preparing it to receive a fertilized egg or shedding its lining if pregnancy does not occur. Both of these cycles occur concurrently and influence each other (Reed & Carr, 2000; Thiyagarajan et al., 2025).

B. Ovarian Cycle

The ovarian cycle can be divided into two main phases, known as the follicular phase and the luteal phase, with ovulation occurring in between (Thiyagarajan et al., 2025). The follicular phase on average spans days 1-14 of the menstrual cycle (Reed & Carr, 2000). The follicular phase begins on day 1 of the menstrual cycle which is characterized by the first day of menstrual bleeding and ends with ovulation. During this phase, follicles which contain egg cells grow and mature in the ovaries. Around day 5 of the cycle, the follicles begin to approach the surface of the ovaries. The follicular phase ends around day 14 when the mature and dominant form of the follicle ruptures to release an egg into the fallopian tube for fertilization by sperm cells, which is known as ovulation (Reed & Carr, 2000). When ovulation concludes, the ovarian cycle will enter the luteal phase where the rest of the ruptured follicle remains in the ovary and becomes the corpus luteum. The corpus luteum secretes hormones which aid the growth of the **endometrium**

of the uterus. If the egg cell is not fertilized, the corpus luteum will degenerate and a new cycle will commence (Reed & Carr, 2000).

C. Uterine Cycle

The uterine cycle includes three main phases: menses, the proliferative phase, and the secretory phase (Reed & Carr, 2000; Thiyagarajan et al., 2025). Menses is the first day of the menstrual cycle and is categorized by the shedding of the endometrium lining which causes cyclic vaginal bleeding lasting, on average, between three to five days (Reed & Carr, 2000). Once menses has ceased around day 5, the endometrium lining begins to regrow during the proliferative phase. The growth of the endometrium lining prepares the uterus for implantation of a blastocyst if the egg cell is fertilized (Reed & Carr, 2000). The secretory phase occurs around day 15 after ovulation and is characterized by the further preparation of the endometrium for possible implantation. It typically lasts from day 15 to 28 of the cycle. During this phase, the corpus luteum produced in the ovarian cycle generates progesterone which prepares the endometrium for possible implantation of a fertilized egg. If the egg cell is not fertilized, the lining of the uterus is shed and menses occurs again, starting the cycle over (Reed & Carr, 2000).

D. Menstrual Cycle Phases and Hormonal Regulation

a. Key Hormones and Their Roles

Spanning an average of twenty-eight days, with normal variations between twenty-one and thirty-five days, the menstrual cycle consists of four distinct phases: menstruation, follicular, ovulatory, and luteal. The hormonal activity across four distinct phases are all led by the hypothalamic-pituitary-gonadal (HPG) axis (Dwyer & Quinton, 2019). This neuroendocrine system begins in the hypothalamus, a brain region that secretes gonadotropin-releasing hormone (GnRH) in a pulsing fashion. GnRH stimulates the **anterior pituitary** to release two key

gonadotropins: follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These gonadotropins then act on the ovaries to regulate the production of sex steroids, mainly **estradiol** (the primary estrogen), progesterone, and testosterone (Johnson, 2018). Their rising and falling levels create a feedback loop that drives the menstrual cycle's changes.

Of these sex steroids we will be focusing on two in particular: estradiol and progesterone. To begin, estradiol serves as the dominant regulator. Among the three primary estrogens, **estrone** (E1), estradiol (E2), and **estriol** (E3), estradiol serves as the most biologically active form during reproductive years (National Academies of Sciences et al., 2020). Its potency stems from having the strongest binding affinity for estrogen receptors, particularly in reproductive tissues (Hall et al., 2001). Primarily produced by growing ovarian follicles, it exerts both negative and positive feedback effects on the HPG axis depending on concentration and the phase of cycle. Progesterone, secreted mainly by the corpus luteum, plays a vital role in cycle regulation and endometrial preparation (Cable & Grider, 2023).

b. Phases of the Menstrual Cycle

The cycle begins with menstruation (days 1-5), which serves as both the conclusion of the previous cycle and the initiation of the new follicular phase. This phase is marked by the extreme decline in estradiol and progesterone, leading to the lowest levels of both sex steroids in the system resulting in the shedding of the endometrial lining (van Santbrink et al., 1995). The decline in sex steroids furthermore removes negative feedback on the hypothalamus, allowing GnRH to stimulate a small increase in FSH secretion from the pituitary (Reed & Carr, 2018).

Rising FSH levels initiate recruitment of twenty primary ovarian follicles, with their granulosa cells responding by producing both estradiol and inhibin B. These inhibins serve as key regulators: inhibin B (produced in the follicular phase) and inhibin A (from the previous

luteal phase) serve as endocrine signals that suppress pituitary FSH secretion without affecting LH secretion. During the follicular phase (days 1-13), developing follicles increasingly secrete inhibin B, which joins estradiol in providing negative feedback to moderate FSH levels and establishing a single dominant follicle selection rather than multiple follicles (Reed & Carr, 2018). By day 5, a dominant follicle emerges, and then begins prepping for follicle rupture and ovulation (Mihm et al., 2011).

The ovulatory phase (around day 14) begins when there is a peak in estradiol levels staying above ~200 pg/mL for roughly 50 hours (Young & Jaffe, 1976). This prolonged high estrogen level triggers an LH surge that causes ovulation - the release of a mature egg from its ovarian follicle into the fallopian tube. At the same time initial rise in progesterone from the preovulatory follicle helps also increase the LH surge and initiates the luteal transition (Reed & Carr, 2018).

During the luteal phase (days 15-28), the ruptured follicle transforms into the corpus luteum, which secretes high quantities of progesterone (peaking at ~25 mg/day) along with estradiol and inhibin A (Reed & Carr, 2018). Progesterone's main roles include preparing the endometrium for potential implantation and suppressing GnRH pulsatility to prevent additional follicular development. Inhibin A further contributes to FSH suppression. Without pregnancy, the corpus luteum regresses after approximately fourteen days. As the corpus luteum regresses at the end of the luteal phase, causing progesterone withdrawals, inhibin A levels decline dramatically, removing this suppression and allowing FSH levels to rise a new causing the start of menstruation, thereby restarting the cycle (Cable & Grider, 2023).

The hormonal changes throughout the menstrual cycle may contribute to various physical and emotional symptoms commonly called Premenstrual Syndrome (PMS). While some females

report symptom patterns that seem to align with certain cycle phases, it's important to note these experiences vary widely between individuals (Romans et al., 2012). Research suggests possible connections between hormone fluctuations and symptoms like mood changes or bloating, though other biological systems and environmental factors likely contribute to PMS (Kwan & Onwude, 2015). These possible connections between hormone fluctuations and menstruation symptoms may explain how menstrual cycle changes might affect other bodily systems.

E. PMS vs. GI Symptoms

Premenstrual syndrome (PMS) symptoms are a set of recurrent physiological and physical symptoms that occur during the luteal phase of the menstrual cycle and may continue to persist into the follicular phase when menstruation begins (Kwan & Onwude, 2015). These symptoms typically include abdominal pain, breast tenderness, headaches, fatigue, bloating, irritability, and cramps (Kwan & Onwude, 2015). Many of these overlap with gastrointestinal (GI) symptoms, which can include abdominal pain, bloating, constipation, diarrhea, nausea, and reflux (Almario et al., 2019). In more severe cases, individuals report experiencing abdominal cramps, vomiting, and epigastric pain (Muhlbacher & Kaczynski, 2021).

Although PMS and GI symptoms are expressed similarly, their etiology and origins differ. PMS-related symptoms are primarily driven by hormonal fluctuations, particularly changes in estrogen and progesterone, which influence fluid retention and pain perception (Rosenfield, 2008). These changes in hormone levels can often lead to psychological symptoms as well, including mood swings, insomnia, and depression (Gudipally, 2023). The decrease in estrogen levels during the late luteal phase and early follicular phase causes the hypothalamus to release norepinephrine, triggering a decline in dopamine, acetylcholine, and serotonin

(Gudipally, 2023). Although the exact etiology remains unclear, the decline in these neurotransmitters appears to lead to the expression of psychological related PMS symptoms (Braverman, 2007).

Gastrointestinal (GI) symptoms include a range of digestive issues that affect the stomach and intestines, often including bloating, abdominal pain, constipation, diarrhea, nausea, and reflux. These symptoms can result from various causes such as dietary habits, stress, gastrointestinal infections, or underlying functional disorders like irritable bowel syndrome (IBS) (Chey et al., 2015). Although GI symptoms are common across populations, studies have shown that women are disproportionately affected, with hormonal fluctuations and psychosocial stressors potentially amplifying symptom severity (Adeyemo et al., 2010). The multifactorial origins of GI symptoms make them challenging to diagnose and treat, particularly when they appear cyclically in tandem with phases of the menstrual cycle.

The overlapping symptoms between premenstrual syndrome (PMS) and gastrointestinal (GI) disturbances, such as abdominal pain, bloating, and nausea, may be explained by shared physiological mechanisms influenced by hormonal fluctuations during the menstrual cycle. The cyclic rise and fall of estradiol and progesterone not only impact reproductive tissues but also modulate gut motility and visceral sensitivity, leading to altered bowel habits and discomfort (Farthing et al., 2015). Elevated levels of prostaglandins during menstruation have been shown to increase smooth muscle contractions in the uterus and gastrointestinal tract, contributing to diarrhea and abdominal cramping. Moreover, these hormonal changes can influence the gut-brain axis, a bidirectional communication system linking emotional and gastrointestinal regulation, which may explain why psychological symptoms such as mood swings and stress commonly co-occur with GI complaints (Enck et al., 2016). Therefore, understanding the interplay between

hormonal cycles and gastrointestinal physiology is critical for improving diagnostic accuracy and treatment strategies for females presenting with cyclical abdominal symptoms.

III. The Gastrointestinal Tract and Gut Motility

The gastrointestinal (GI) tract, also referred to as the gut, is the continuous tube that moves food from the mouth all the way to the anus. This movement, called gut motility, is essential for digestion and nutrient absorption (International Foundation for Gastrointestinal Disorders, 2025). The organs that make up the GI tract are the oral cavity (mouth), esophagus, stomach, small intestine, large intestine (colon), rectum, and anus (U.S. Department of Health and Human Services, 2022). In order to ensure homeostasis in the body, the autonomic nervous system (ANS) controls the nerves found in these gastrointestinal organs. The ANS controls the sympathetic nervous system, the parasympathetic nervous system, and the enteric nervous system, or ENS. These systems are stimulated by the consumption of food and work together to control the movements of the gut to aid in digestion (Wehrwein et al., 2016).

As food enters the mouth and continues through the gut, it is mechanically and chemically broken down into materials that the body is able to use. Any food that remains is first metabolized by gut **microbiota** and then gets excreted as waste (Seeley et al., 2003). A primary function of the ENS is to control **peristalsis**, which moves partially digested food through the GI tract (Patel & Thavamani, 2021). Peristalsis combines muscle contractions closer towards the mouth with muscle relaxation towards the anus in order to propel the **bolus** across the whole length of the gut (Olsson & Holmgren, 2001). The slower rate at which peristalsis occurs gives the digestive organs sufficient time to further break down the bolus to absorb as many essential nutrients as possible (Cleveland Clinic, 2022). Peristalsis works to mix the contents in the

intestines of the GI tract, expose the contents to the mucus layer found in the gut, and push the contents forward (Seeley et al., 2003). Thus, the efficiency of peristalsis greatly impacts the overall health of the gut.

Peristalsis is just one of the many factors that influences transit time, or the amount of time it takes for a food item to travel through the GI tract (Procházková et al., 2023). The different foods found in an individual's diet can increase or decrease transit time. For example, fiber is a carbohydrate that is resistant to digestion and can be either soluble or insoluble in water (Brownlee, 2011). During digestion, soluble fibers are able to absorb water to form a gel-like substance, which slows the movement of food through the GI tract, allowing for more nutrients to be absorbed (Procházková et al., 2023). On the other hand, insoluble fibers increase the bulk of stool, triggering peristalsis and speeding up the movement of food through the GI tract (McRorie & McKeown, 2017). Soluble fibers are found in beans, fruit, and nuts while insoluble fibers are found in wheat, bran, and whole grain products (National Library of Medicine, 2024).

A. Gut Microbial Gas Production

Foods containing high amounts of fiber are linked to an increase in flatulence, or gas released from the digestive system through the anus (Cleveland Clinic, 2025). In order for nutrient absorption to occur, **glycoside hydrolases** must be present in order to break down the **glycosidic bonds** that hold molecules together. These bonds must be broken in order for the large molecules found in the foods that humans consume to become much smaller (Withers & Williams, 2022). It is important to note that these glycoside hydrolases are unable to break down every molecule in all food items that are often consumed. Humans lack the glycoside hydrolases that are needed to break down carbohydrates, such as fiber, and allow for their digestion and

absorption. Fortunately, the gut **microbes** are able to break down the molecules that humans themselves cannot. The microbes of the gut encompass all the microorganisms, including bacteria and viruses, that are present in the GI tract (Donaldson et al., 2016). Gut microbes break down these indigestible molecules through a process called **fermentation**. The gases produced as a byproduct by the gut microbes include hydrogen gas, carbon dioxide, methane, and hydrogen sulfide (Levitt, 1980). Approximately 99% of intestinal gas produced is composed of odorless hydrogen, nitrogen, carbon dioxide, and methane, while the remaining 1% is composed of odiferous gases that have a distinct smell (Mutuyemungu et al., 2023). Gastrointestinal symptoms, such as flatulence, constipation, pain in the lower abdomen, and bloating, are often a result of abnormalities to the production of gas in the gut and the transit time for these gases (Serra et al., 2001).

The health of the gut is impacted by diet as well as gut microbe diversity. Researchers believe that an individual's gut microbial diversity is due to genetics, environment, and early exposure to these microbes. In healthy individuals, the gut is populated by a broad variety of microbial species (The Human Microbiome Project Consortium, 2012). This is why the **dysbiosis** of the gut is linked with many diseases (Sidhu & van der Poorten, 2017). Additionally, the different organs of the gut have different levels of bacterial diversity. For example, the colon has more diverse bacteria than the small intestine does. This is due to differences in acidity, chemical and nutrient gradients, transit time, and the compartments and folds that make up each different location (Donaldson et al., 2016).

B. Prior Research About Relationship Between Menstrual Cycle Hormones and Gut Motility

Prior research between menstrual cycle hormones and gut motility is scarce, with few papers explicitly examining gastrointestinal functioning in relation to the menstrual cycle in young adults, as well as changes in the gut **microbiome** throughout the menstrual cycle. In a cross sectional study amongst emerging young adults, six hundred participants completed a survey that included GI assessment scales, an abdominal pain scale, and a health anxiety measure. Participants were grouped via their menstrual cycle phases based off of self-reported data. Multivariate analyses of covariance were conducted to identify if there were any relationships between the participants' menstrual cycle phase and participants' scores on the symptom scales. The results of this study stated that there were no significant differences found, and that GI symptoms measured were not dependent on menstrual cycle phases. However, the study found that participants' pain in the hypogastric region of the abdomen was significantly higher during the menses phase as opposed to the early-luteal phase and premenstrual phase. Furthermore, higher GI-symptom and pain levels were found as health anxiety of the participants increased, which the researchers thought could explain observed-sex differences in GI pain (Mendelson et al., 2023).

Another study reveals loose stool consistency in the early menstrual period in comparison to the midcycle in six out of the seven employed participants (Simmons et al., 1988). Overall, studies investigating GI transit time throughout the menstrual cycle produce contradicting results. For example, some studies conclude that there is an increase in transit time during the luteal phase, while others claim that there is no change in transit time during the luteal phase (Siddiqui et al., 2022). Furthermore, there have been studies done measuring the relationship of

sex hormones found in the menstrual cycle on the gut microbiome. For example, in one study, estrogen, specifically estradiol, was found to activate pathways for proinflammatory cytokines (Yoon & Kim, 2021). Other studies also show that GI transit duration has been shown to vary according to menstrual cycle, pregnancy, and postpartum (Meleine, 2014).

C. Limitations to Current Research:

While the literature landscape points to a correlation between abnormal gastrointestinal symptoms and the menstrual cycle, current research has been limited by research populations and methods used to measure gastrointestinal symptoms. The majority of studies only assess populations with IBS and whether IBS systems are exacerbated during certain phases of the menstrual cycle. For example, in a cross-sectional study conducted on 102 IBS patients, there was a statistically significant increase in reports of symptoms of diarrhea during the menstrual phase compared to the luteal and follicular phase and symptoms of constipation during the luteal phase compared to the menstrual and follicular phase (Pati et al., 2021).

Additional limitations include the current methods to quantify gastrointestinal symptoms. Along with surveys, more objective methods exist to quantify gastrointestinal symptoms such as the hydrogen breath test and radiopaque markers (Bharucha et al., 2019; Sciarretta et al., 1994). The hydrogen breath test is currently used to diagnose several common digestive diseases, such as IBS and lactose intolerance. This method requires participants to fast for eight to twelve hours prior to drinking a sugar solution. In patients with normal gastrointestinal function, the sugar solution will be metabolized in the small intestine. The limited remaining amount of undigested carbohydrates will reach the large intestine, which is eventually digested by anaerobic gut microbiota that produce hydrogen as a byproduct of metabolism. High levels of hydrogen can

indicate carbohydrate intolerances or an excess of small intestine bacteria (Ghoshal, 2011). In addition to diagnostic testing, hydrogen breath tests can also be used to measure transit time. The time from consumption of the sugar solution to the detection of hydrogen can be used to determine orocecal transit time (Sciarretta et al., 1994). However, there are several limitations to this method. First, the signal to noise ratio is poor because the concentration of hydrogen is only 20 ppm. The sensors also aren't specific to hydrogen, so there is the potential for interference from metabolites produced by oral microbes. Measurements are also dependent on many confounding variables such as breath velocity (Ghoshal, 2011).

Another method available to track gastrointestinal transit time is radiopaque markers. In a study measuring transit time during the luteal and follicular phase, twenty-one females ingested twenty radiopaque markers and received X-rays on the fourth and seventh days after ingestion. Females in the luteal phase had a mean colonic transit time that was 20.3 hours longer than females in the follicular phase (Jung et al.). Radiopaque markers can be utilized through several different methodologies which may include ingesting one or multiple types of markers, ingesting markers on day one or over several days, and receiving X-rays on one or multiple days. However, methods that require multiple X-rays are not used often in clinical practice because of the risk of radiation exposure. The most common form of implementation involves ingesting markers over the course of several days and receiving one X-ray. Transit time is thus operationalized as the number of markers still present in the abdomen, with all the markers being present as the longest possible transit time. However, this methodology introduces several systematic errors because it assumes markers are ingested continuously instead of in one bolus each day and that the markers have reached steady-state when the X-ray is taken. This method is

also inappropriate for patients with fast transit time who will have eliminated all markers prior to the X-ray (Bharucha et al., 2019).

Despite these existing mechanisms, no longitudinal study has been conducted on gut transit time over the menstrual cycle. Existing methods often require participants to stay in lab to receive breath analysis, or return to take X-rays (Bharucha et al., 2019). Therefore, we used a novel tool, the Smart Underwear device, to measure gut microbial gas production as a proxy for intestinal transit time.

D. The Smart Underwear Device

The Smart Underwear device is a data-logger that measures the frequency, volume, and composition of flatus. The device can be adhered to the exterior of underwear using a snap or commercial tape, which allows it to be worn non-invasively over several days. Thus, multiple days of data can be collected continually without participants needing to go into a lab or take X-rays. The device is equipped with coin cell batteries, gas sensors, temperature and humidity sensors, an **accelerometer**, and a microcontroller. The electrochemical sensors can be adapted to measure several different biomarkers in the flatus such as hydrogen (ppm), hydrogen sulfide (ppm), and nitric oxide/ nitrogen dioxide (ppm). For our study, the electrochemical sensors in the Smart Underwear device only measure hydrogen (ppm). The device has a significantly higher sensitivity for detecting hydrogen in the flatus, allowing for more precise measurements compared to the hydrogen breath test (Botasini et. al, 2025). Transit time is calculated by comparing when food is consumed (meal times) to the time gas is detected.

E. Hypothesis

We hypothesized that we will detect increased gut microbial gas production during menstruation, when estradiol and progesterone are lowest and transit time is fastest, using the Smart Underwear Device as a proxy for intestinal transit time.

Chapter 2: Methods

I. Enrollment and Recruitment:

The study was designed to track flatus during several points of the menstrual cycle. Nineteen participants were initially enrolled in the study. Fourteen participants completed the full study and returned the device. No interventions were prescribed; thus, the study was purely observational.

Recruitment for the study was primarily conducted through flyers placed on the University of Maryland, College Park campus. Flyers included a QR code linked to a Qualtrics form with several eligibility questions that assessed whether the participant had non-symptomatic gastrointestinal function, a regular menstrual cycle, and normal hormone levels. To have qualified for the study, participants had to experience at least five flatus per day, experience bloating, diarrhea, constipation, flatulence, cramping, and/or other gastrointestinal symptoms when on their period, not have been currently taking antibiotics, and can not have been diagnosed with any gastrointestinal disorders including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, etc. In addition, participants had to have a monthly period with an average menstrual cycle of twenty-three to thirty-three days, and have been tracking their period. Participants must also have had normal hormone levels and thus could not have been diagnosed with primary ovarian

insufficiency, a thyroid dysfunction, Cushing's syndrome, congenital adrenal hyperplasia, Asherman's syndrome, polycystic ovary syndrome (PCOS), uterine fibroids, endometrial polyps, premenstrual dysphoric disorder, and/or adenomyosis or have been on hormonal birth control, including emergency contraception, in the last three months. Descriptive data were also collected on this form which included whether the participant had taken antibiotics in the previous three months, was taking any probiotic/prebiotic supplement, experienced any unexplained weight loss or gain, experienced frequent nausea, vomiting, and stomach pain, had a recent stool type change, or were taking any fiber supplements. Answers to these questions did not determine eligibility.

II. Initial Meeting:

Participants received a Smart Underwear device with verbal and written instructions for wearing the device. Attachment of the Smart Underwear device to underwear was demonstrated on a mannequin. In addition, participants were instructed not to wear the device while swimming, showering, and other situations in which the device might become wet, or when passing through security checkpoints (airports, courthouses, ports of entry). Participants were instructed not to tamper with the device and to keep it away from children. The participant continued normal activities while wearing the Smart Underwear device.

Participants were also given a personal calendar (See Figure A1 in Appendix) of when to wear the Smart Underwear that was tailored for each participant based on the start and length of their menstrual cycle. Participants were asked to attach the Smart Underwear device to their underwear using a snap for all waking hours while dressed for Days 1 to 3, 12 to 14, and 21 to 23 of their menstrual cycle (Figure 1). These days were selected to help evaluate results over the varying phases of the menstrual cycle during different hormonal fluctuations. On the days

that the Smart Underwear was worn, the participant was asked to fill out a food journal documenting their meals, including ingredients in the meals they ate and when they ate that day. Food logs were recorded on a document that was provided for the participant to fill out (See Figure A2 in appendix).

On days 12, 13, and 14, participants were asked to take a luteinizing hormone (LH) test upon waking up to determine if they were ovulating. Three LH test strips and urination cups were provided to each participant, in addition to three extra test strips and urination cups. Verbal and written instructions were given to the participant on how to take the test. Participants were instructed to record the LH test results on the wearing calendar and to wear the Smart Underwear regardless of the results.

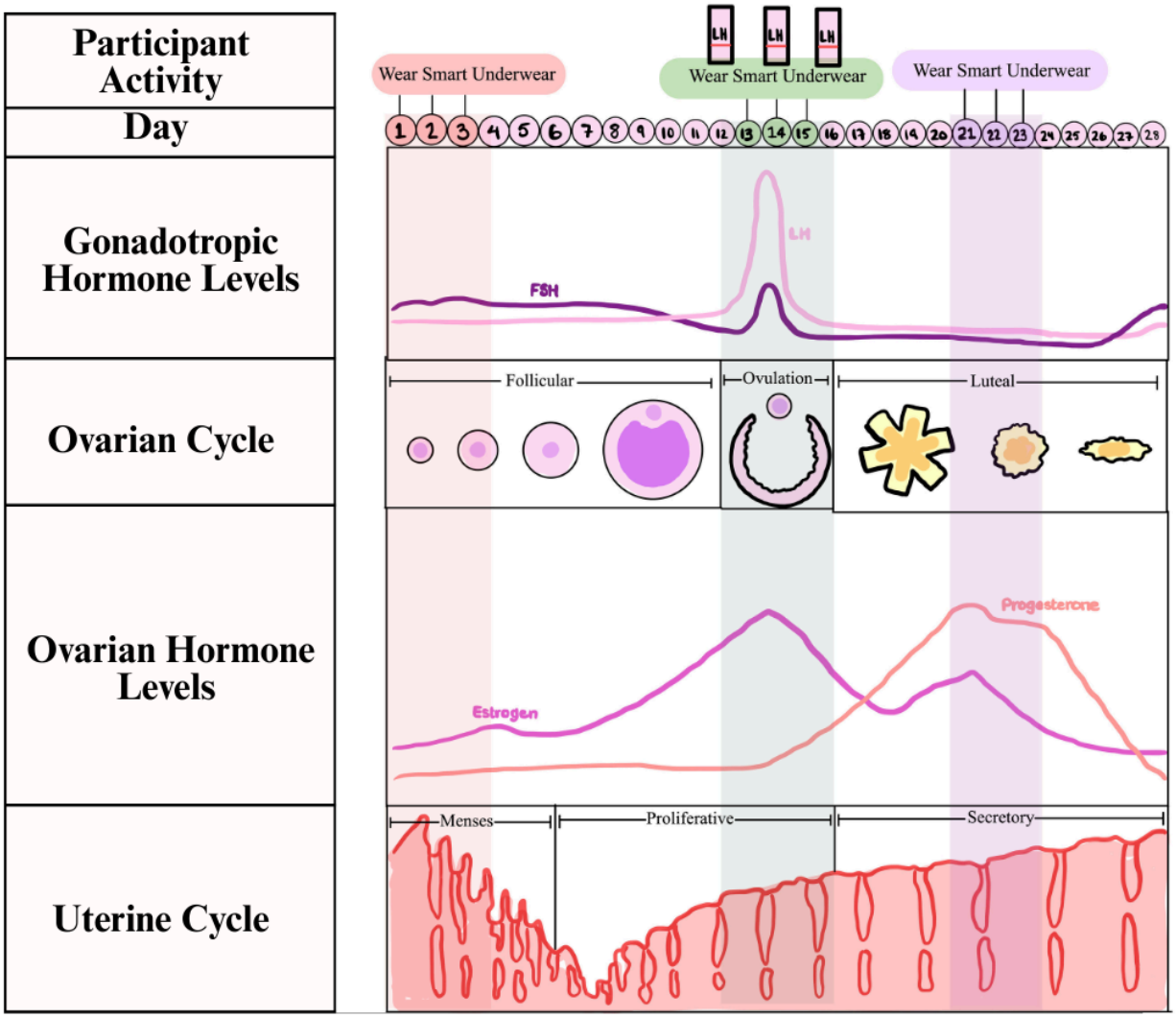

Figure 1: Participant Activity Over the Phases of the Female Reproductive Cycle

III. Final Meeting:

After completing the study, participants were expected to return their Smart Underwear device, wearing calendar, and food journal. Participants were compensated for participating in the study ($50 Tangocard digital gift card or $50 cash). When participants were compensated with cash, they also signed a paper receipt as confirmation. If the participant returned the device, regardless of the number of wearing days, compensation was provided.

IV. Data Analysis

After all devices were returned, the data was extracted. The data was extracted from the sensor where two types of "blocks" are retrieved. Long blocks contained detailed sensor data, such as specific measurements collected when the device detected a signal, and short blocks contained baseline or ambient data, which were simpler and recorded less frequently (every ten minutes). The long block included data from the gas sensor, temperature sensor, and accelerometer. The gas sensor measured hydrogen, allowing us to determine when a flatus occurred. Both the accelerometer and temperature sensor developed an understanding of whether the participant was wearing the device or not based on their body temperature (temperature sensor) and movement (accelerometer). From that information, a wearing probability was developed to understand the likelihood that the participant was actively wearing the device at any given time, increasing data accuracy and reliability. When there was constant movement, the accelerometer signaled a 1; otherwise, it signaled a 0 if there was no movement. Likewise, the closer the temperature is to the body temperature, a higher probability score is assigned to determine if the device has been worn.

Finally, Excel was used to export the data through a csv file and to create the graphs. A graph depicting flatulence over time was then created by plotting the sensor's reading against time (in hours). Following that, a graph illustrating the probability of wearing over time was generated by plotting the probability of wearing as a function of time (in hours). The final graph depicts the Microbiome Activity Index (MAI), a quantitative metric designed to estimate the metabolic activity of gut microbiota by analyzing flatus frequency and hydrogen concentration. This is an empirical and arbitrary number that was fabricated based on previous studies as a way to condense both flatus number and intensity in a single number for comparison (Botasini et. al,

2025). To create this graph, we computed the first derivative of the sensor signal to measure the rate of change in gas concentrations over time. Using the absolute value of this derivative, rather than the raw signal, allowed for more accurate identification of flatus events by highlighting rapid shifts in gas levels. We then calculated the Microbiome Activity Index as the total number of time points where the derivative exceeded a dynamic baseline threshold, enabling more responsive and precise monitoring of microbiome-related activity. Using this derivative, a formula was applied to count the number of points exceeding 4000 (the defined threshold for the MAI) during each phase of the cycle. This threshold was predetermined based on prior experiments, providing a validated rationale for our data processing choices and allowing us to maintain consistency across analyses. Finally, the MAI values were plotted over time to visualize microbiome activity. All three graphs were used to analyze and compare trends across the different phases of the menstrual cycle.

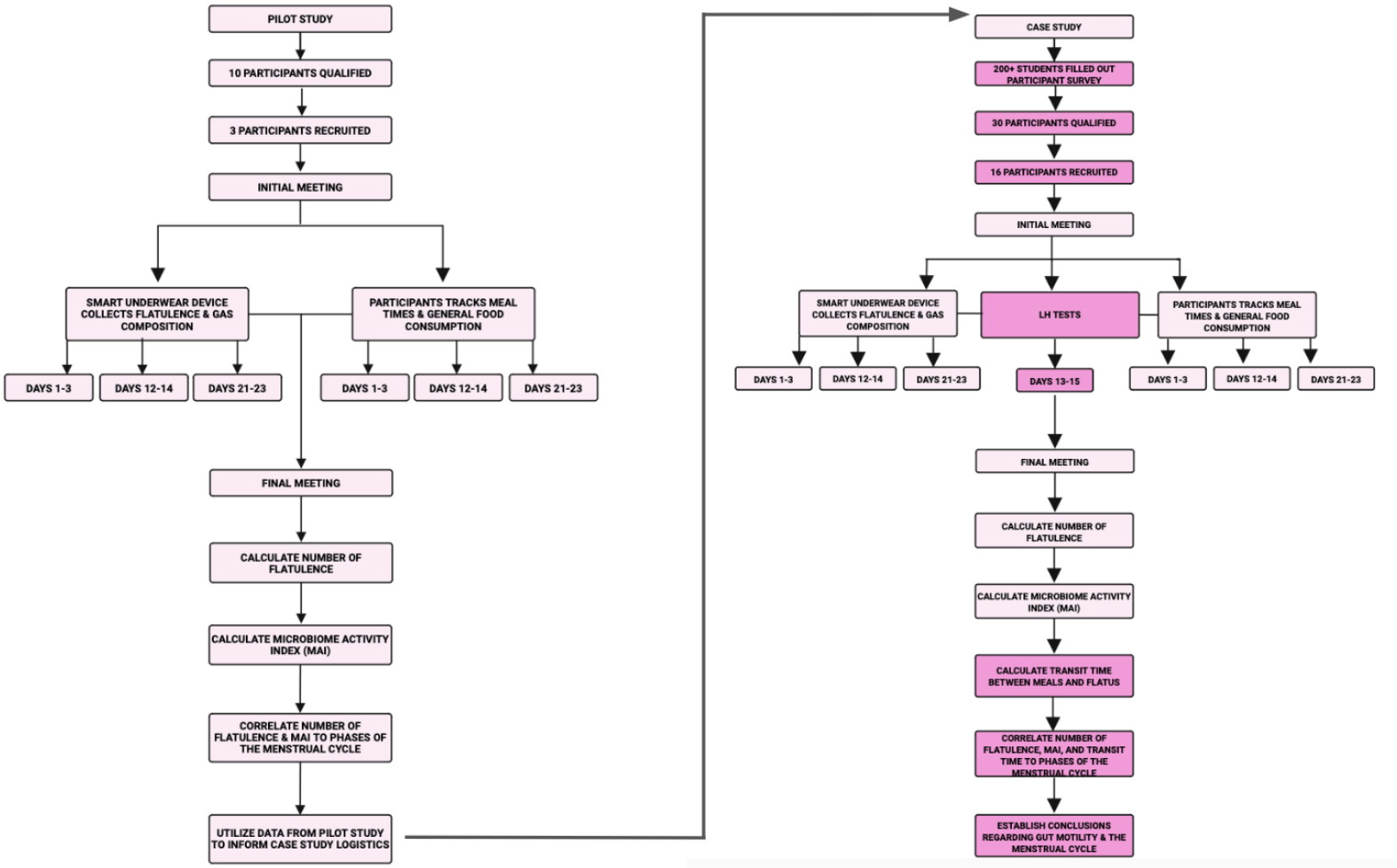

Figure 2: Pilot and case study methodology

Chapter 3: Results

I. Collected Data

The objective of this study is to investigate changes within the gut health across different phases of the menstrual cycle by analyzing functions in gas levels and microbiome activity. Nineteen participants enrolled into the study, with only fourteen devices returned. Data was collected from participants over nine months with each participant wearing it for a total of nine days during a month, capturing gas levels and microbiome activity across the follicular, ovulation, and luteal phases. This data allows us to analyze flatulence over time for each

participant in addition to knowing the probability of wearing the device over time. In addition to analyzing flatulence and probability of wearing over time, the Microbiome Activity Index (MAI) was calculated as a way to compare the outcomes of the different flatus profiles throughout the menstrual cycle. The MAI is defined as a novel metric that measures the metabolic activity of gut microbes by analyzing flatus composition. This index provides a quantitative measure of microbiome activity across different phases of the menstrual cycle. Finally, the last metric collected was the LH strips results during the ovulation phase to determine if the participant was ovulating. These results provided a reference point for aligning microbiome activity data with hormonal changes, ensuring accurate phase identification.

The collected data was then visualized through various graphs to identify trends across the menstrual cycle. Initial findings suggest that microbiome activity varies significantly across phases, with heightened activity during ovulation. Our analysis revealed that higher concentrations of flatulence peaks occurred during the ovulation phase as shown in Figure 3 and

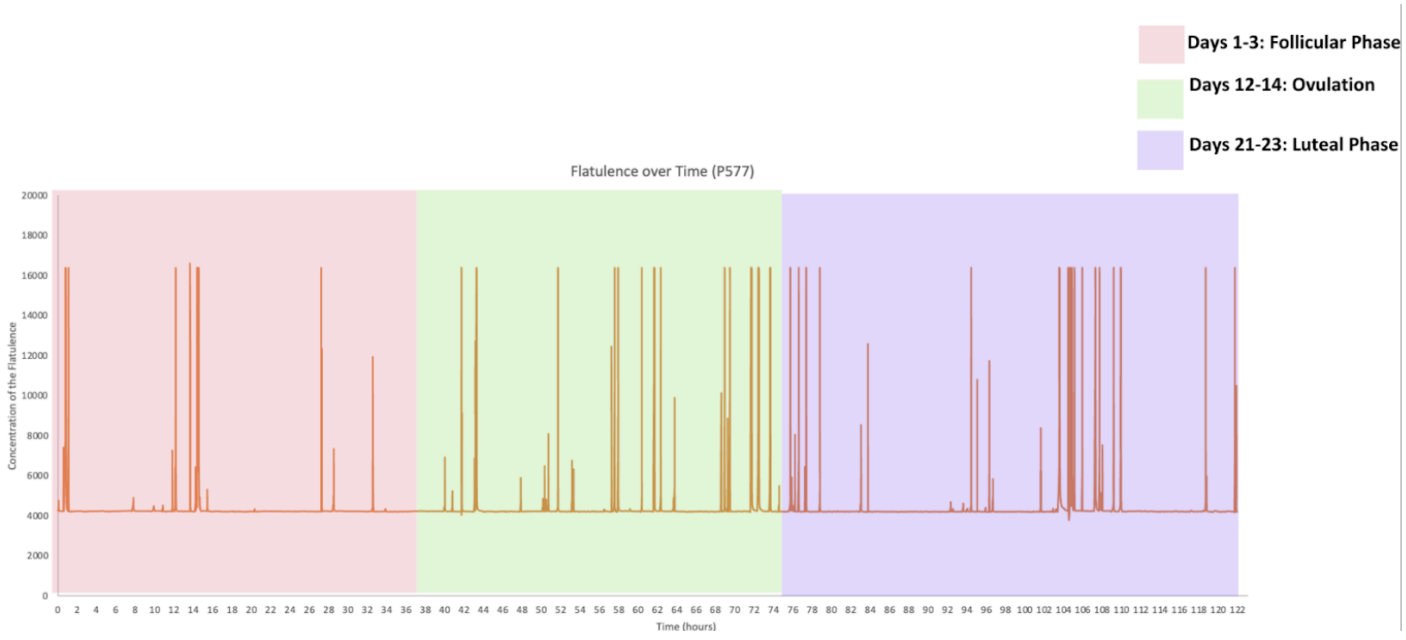

Figure 4.

Figure 3: Flatulence over Time Graph for Participant 577

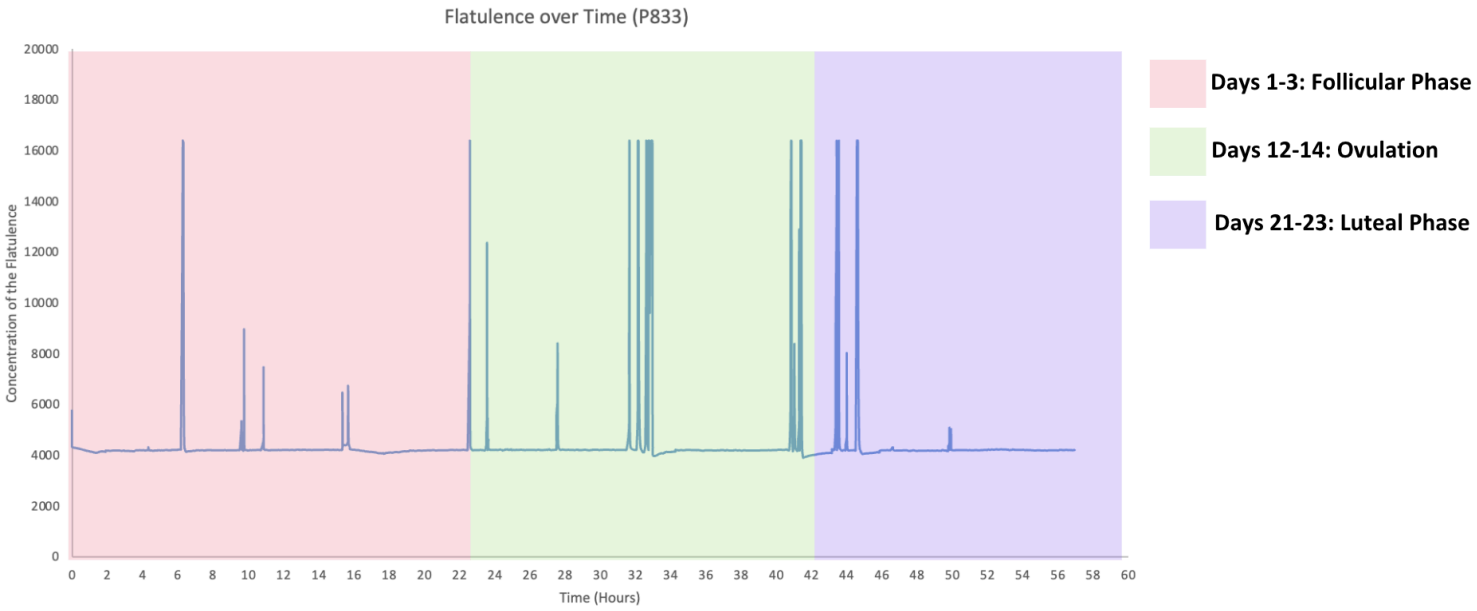

Figure 4: Flatulence over Time Graph for Participant 833

This trend was confirmed by the MAI graph as shown in Figure 5 and 6 as the MAI was significantly higher during ovulation compared to other phases. For Participant P833, as shown in Figure 6 their LH strip tested positive, confirming that they were ovulating and further validating the observation that the MAI was elevated during this phase.

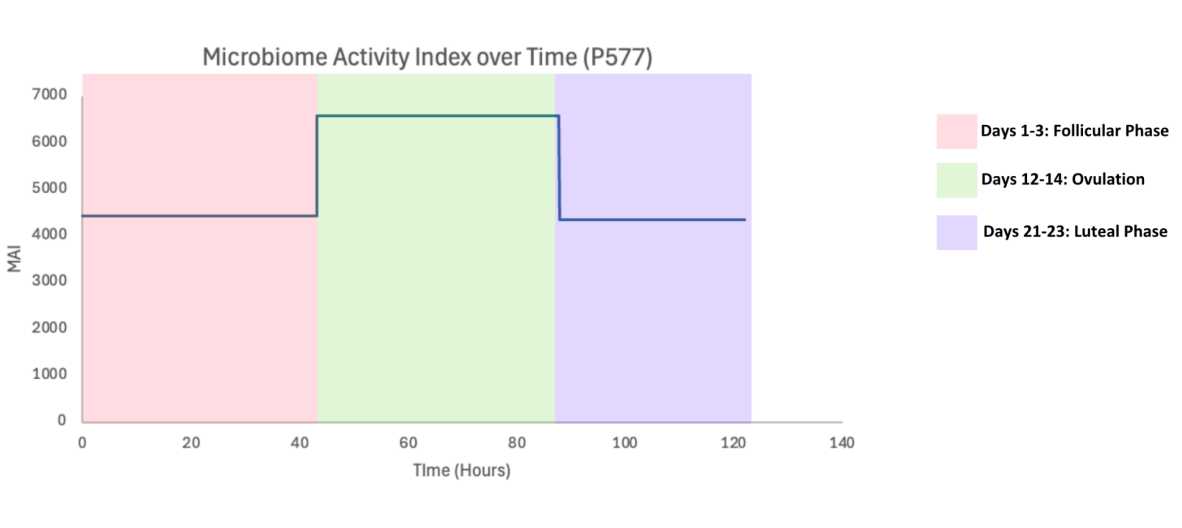

Figure 5: Microbiome Activity Index over Time Graph for Participant 577

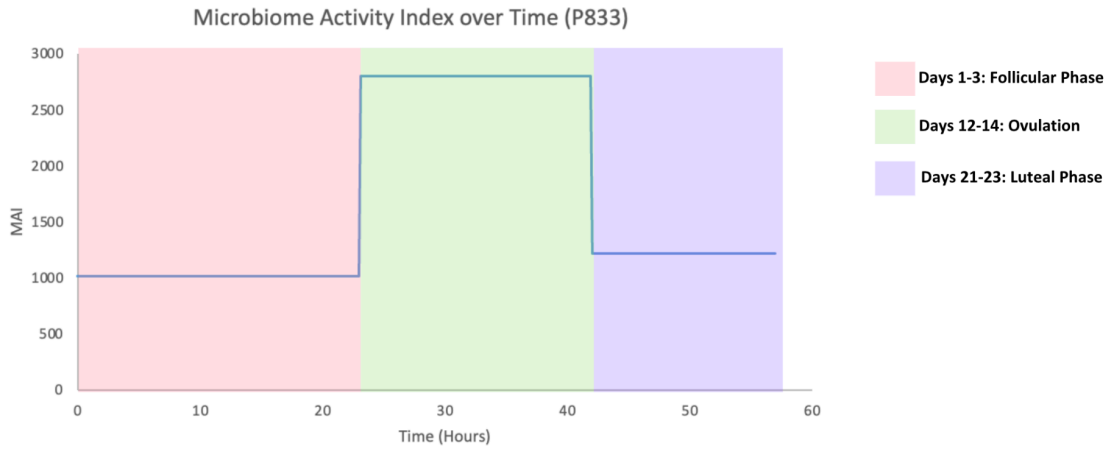

Figure 6: Microbiome Activity Index over Time Graph for Participant 833

Although many participants' MAI was higher during ovulation, this varied differently depending on the participant. As shown in Figure 7, the MAI is compared for all participants across three distinct study phases: Follicular Phase, Ovulation, and Luteal Phase. Each box represents the interquartile range (IQR), with the median marked by a horizontal line. The whiskers extend to show the range of the data, excluding outliers, which are displayed as individual points. The plot reveals that the median MAI increased throughout Phase 1, Phase 2,

and Phase 3, suggesting that microbiome activity increased through the different phases. While the median MAI values differ across phases, Follicular Phase and Luteal Phase exhibit a noticeably wider range of values compared to Ovulation, indicating greater variability in microbiome activity among participants during those times. This can be due to the discrepancy of the participants exact cycle as we lacked information on the exact length of each participant's cycle, so many participants may be on their follicular phase when taking data for the luteal phase.

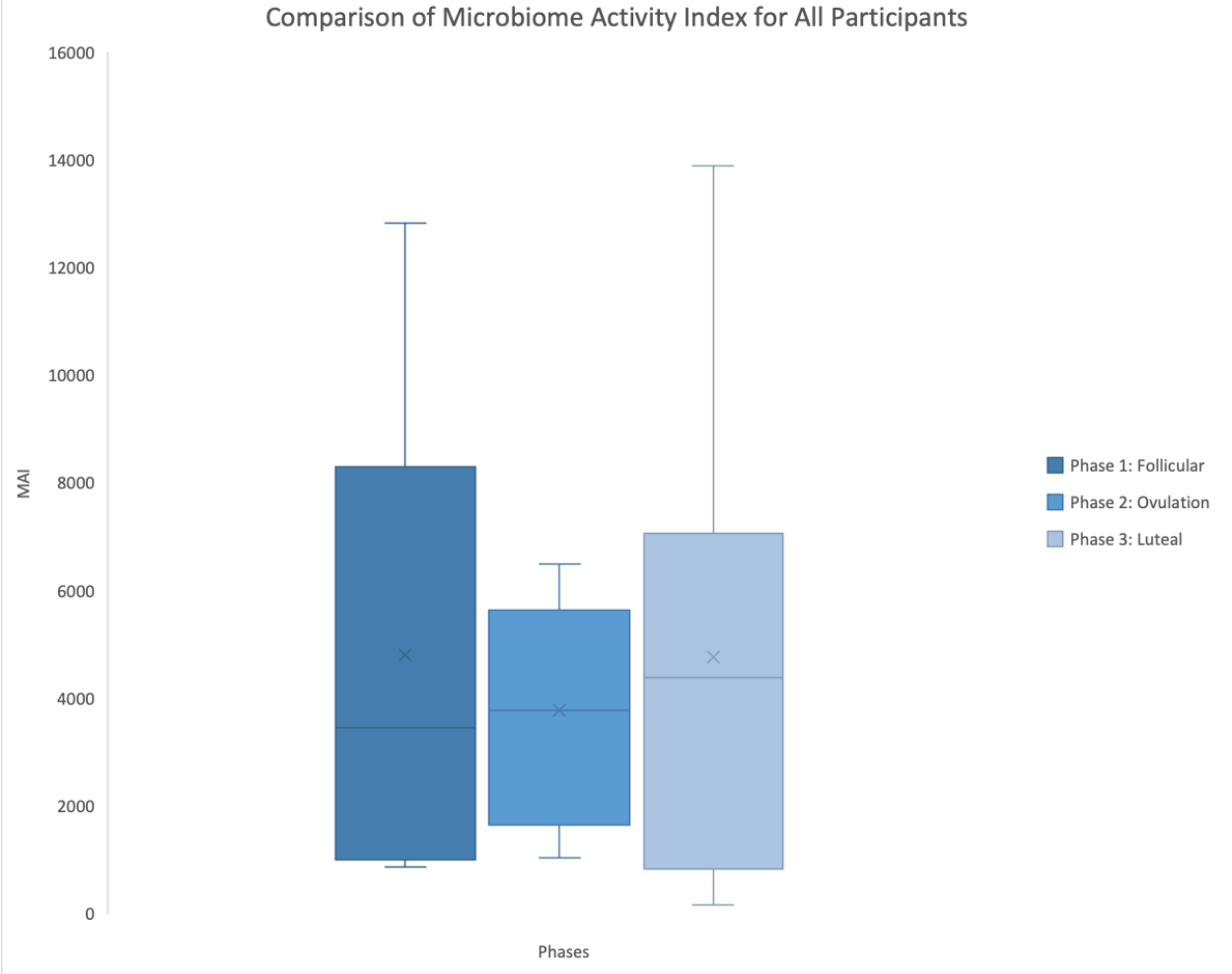

Figure 7: Comparison of Microbiome Activity Index (MAI) Across the Three Phases for All Participants

These findings represent the first time microbiome activity has been measured throughout the menstrual cycle, underscoring the potential for future studies to build on this work and explore these patterns in greater detail. Our study highlights the feasibility of using the Smart Underwear device throughout a menstrual cycle and continuous monitoring to capture microbiome fluctuations. There were several limitations in this study. Many participants did not wear the device on the designated days and instead wore it on different days, preventing us from accurately aligning their data with the intended phases of the menstrual cycle. Since we lacked information on the exact length of each participant's cycle, we were unable to reliably estimate which phase they were in during data collection. As a result, this misalignment limited the amount of usable data, reducing the sample size and affecting the reliability of the conclusions drawn about microbiome activity across different phases of the menstrual cycle. Future research could incorporate larger sample sizes and statistical analyses to validate these observations and refine the measurement techniques as talked about in the next section.

During days 12 to 14 of the menstrual cycle, we used LH strips to predict ovulation . However, most participants recorded negative results as shown in Figure 8. This discrepancy may be attributed to the natural variability in menstrual cycle lengths and ovulation timing, which differs among individuals. While day 14 is typically considered the average day of ovulation, many people ovulate earlier or later depending on their unique cycle patterns. Additionally, the LH surge can be brief, and can be easily missed if testing is done only once per day (Higuera, 2020). Other factors, such as overhydration diluting urine samples, the sensitivity of the LH strips, and potential hormonal imbalances, may have also contributed to the negative readings. These results suggest that a broader testing window and more frequent testing may be necessary to reliably capture the LH surge and confirm ovulation timing. Finally, many

participants forgot to test consistently on the allocated days, which may have affected the accuracy of LH strip results.

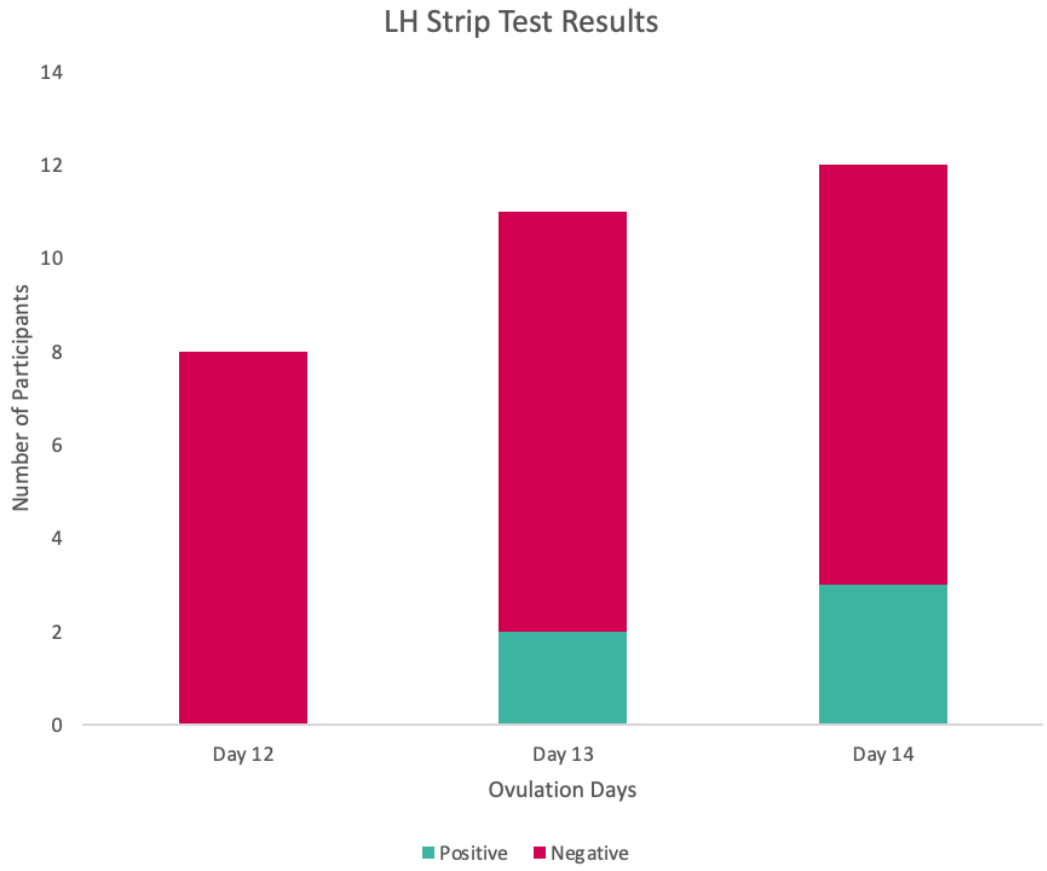

Figure 8: LH Strip Test Results Over Three Days

II. User Experience Survey Results

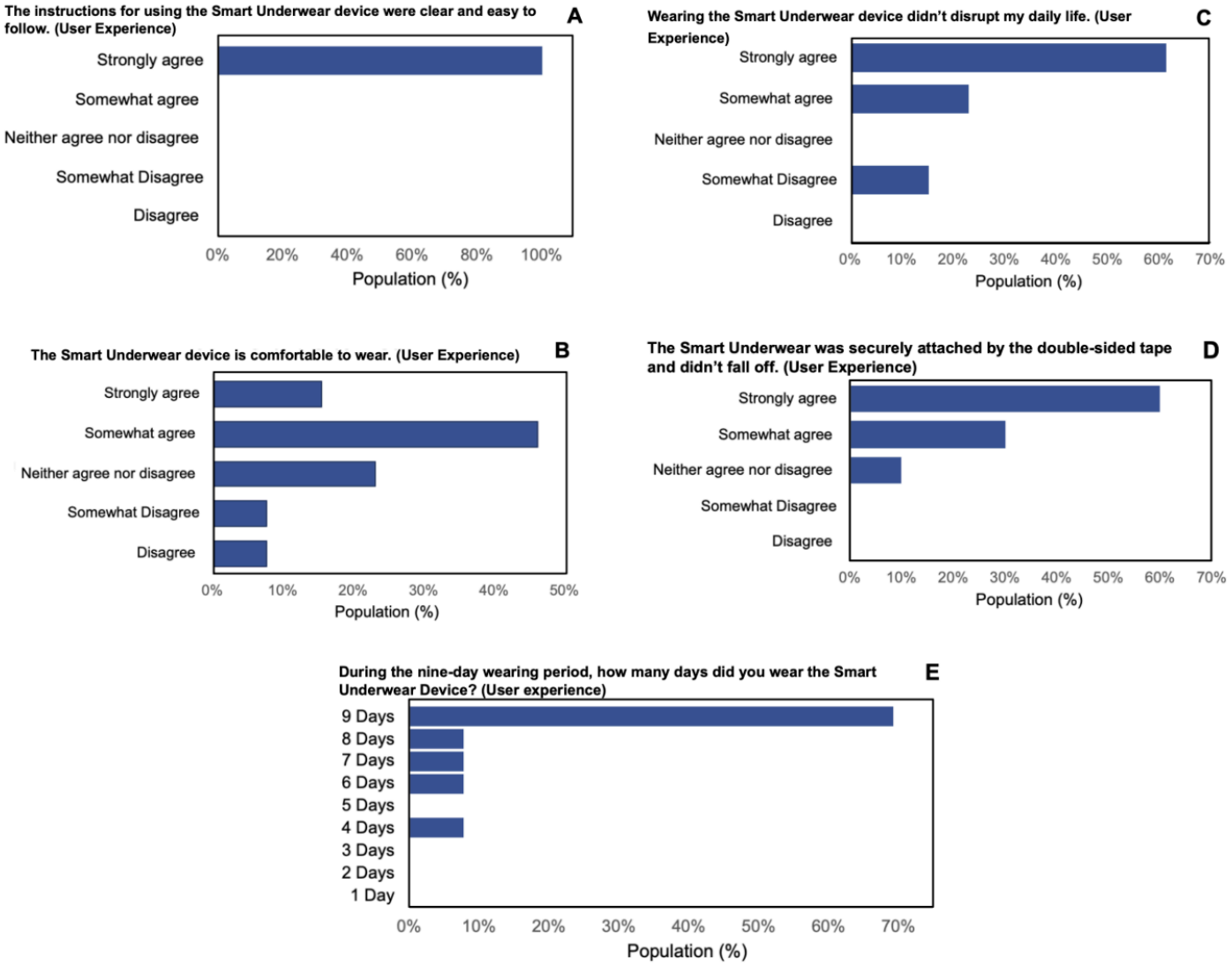

Figure 9: User Experience Survey Results

The User Experience survey results demonstrated that participants generally had a positive experience wearing the device throughout the Team BELI Study. Most found the study instructions straightforward and easy to follow, and comfort ratings were consistently high, indicating the device was wearable for extended periods. Feedback also noted that the design allowed everyday use without significant inconvenience. In discussions with participants who reported discomfort, many preferred a smaller or less rigid/square device. According to Figure

9E, most participants completed the 9-day wear period as intended, though a small number discontinued early. Written feedback suggested that early study dropouts were mainly due to forgetfulness or external logistical factors, rather than issues related to the device itself. These findings suggest strong comfort and usability of the overall Smart Underwear devices.

Chapter 4: Discussion

I. Conclusions:

Team BELI utilized The Smart Underwear device to create a novel research project that monitored gut motility across the menstrual cycle. By implementing The Smart Underwear device we were able to actively record flatus composition and number of flatus over nine days. Comparing the results over three different times in the menstrual cycle, we were able to assess if any patterns or similarities were present. Although initially there was a trend of participants having a higher Microbiome Activity Index (MAI) during ovulation, this varied participant to participant, and as a result, no clear or consistent trend was observed, and the data did not support our initial hypothesis. The Smart Underwear device measured gut microbial gas production as a proxy for intestinal transit time. The data we collected depicts an increase in microbiome activity and quantity of flatus in the three days that represents the ovulation phase. While we currently have insufficient data to assess the transit time of individual participants, this preliminary data successfully supports future studies in this area of research.

II. Novelty:

In past research investigators used the hydrogen breath test in order to measure transit time and to diagnose common digestive disorders. However, The Smart Underwear device was

created to be able to quantify the hydrogen levels in an individual's flatus. The sensors in the Smart Underwear device are more sensitive and are also able to accurately detect the measurements of hydrogen in flatus (MAI). Compared to the hydrogen breath test, the Smart Underwear device has a significantly higher sensitivity for detecting hydrogen in flatus, which allows for more precise measurements. The device is less involved and time consuming than the hydrogen breath test and can track hydrogen gas production across several days, as it has a battery life of approximately 12 days. Smart Underwear can be used as a tool to observe changes in GI symptoms and changes in transit time throughout the menstrual cycle in females, while they go about their daily lives. This would not be feasible using the hydrogen breath test, as it involves hours spent in a lab and radiopaque markers would require numerous X-rays, exposing participants to excess radiation.

III. Project Weakness:

We frequently ran into problems with participant attendance for our study. Participants often did not attend meetings, did not wear the device on the indicated days, or failed to complete the wearing calendar, and thus could not be included in the data analysis. In efforts to encourage participant engagement moving forward, participants were asked to fill out a feedback survey concerning the device's comfortability at their final meeting. Additionally, during the case study we sent reminder emails based on each participant's wearing calendar one day before each wearing period.

While participant feedback indicated an overall positive experience, some suggested reducing the device size and modifying its shape to improve comfort. Additionally, future direction in enhancing the Smart Underwear device could focus on extending the battery.

Increasing battery life would minimize participant burden and reduce the risk of data loss due to power depletion. It would also allow future research to build off of this study, having participants wear the device for the full duration of their menstrual cycle. Furthermore, throughout the study we received questions regarding the device and LH strips, or forgetfulness when it came to wearing the device. A potential solution would be to increase our communication with the participants by communicating with them through reminder phone calls or text messages rather than email. Another alternative would be to create a live chat where they can reach us with questions.

Another problem we faced was with the accuracy of the LH strips and timing of the menstrual cycle. Most of the LH strips came back negative, which indicates that the participant was not actively ovulating. In future studies, testing for LH over more days before and after day 14 in each participant's menstrual cycle would be advised. In an ideal setting we would utilize hormone testing, specifically drawing blood, in order to get an exact level of the hormones in the participants body each day. This would be the most accurate way to determine which phase of the cycle the participant is entering, and if they consistently have steady levels. However, we did not have access to the necessary tools for blood hormone tests and we wanted to avoid invasive tests so as not to discourage participation.

Additionally, we did not have participants follow a strict diet for the duration of the study, meaning that our results could have been influenced by what the participants were eating. For example, if participants were consuming foods resistant to digestion, then their gut microbes were able to utilize these carbohydrates for fermentation, resulting in gas production. The foods of concern would be of high FODMAP, which stands for fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. Typically a FODMAP diet is followed by

individuals with IBS in order to minimize uncomfortable gastrointestinal symptoms due to the gut microbial gas production caused by FODMAP foods (Böhn et al., 2015). Ideally, participants would have followed a low-FODMAP or similar diet, which would have ensured that any gas production was due to hormone levels and not food content.

IV. Future Directions:

Investigating female health in this project was of the utmost importance to the team. Our intention for this research was to start the conversation about a potential connection between menstrual symptoms and gastrointestinal transit time. As stated earlier, females are often underrepresented in clinical research. Thus, it was of importance to our team to increase the number of females participating in gut health research. Therefore, we sought to specifically enroll females in this study and focus directly on female reproductive health in our research. Specifically, increasing the amount of research of females during their period and how the hormones of the menstrual cycle can affect disorders of the gut. One of the reasons we are so passionate about our research is because females have historically been left out of research because of their hormone cycle. This has led to medications and developments that have not been tested on women, and could cause adverse effects. Females are significantly overdiagnosed with digestive disorders because of the lack of research into how the menstrual cycle can impact the gut microbiome (Patient Safety Learning, 2022). The results of our research can spread awareness about the impacts of false digestive disorder diagnoses in females while contributing to a significant knowledge gap. In future studies, we would increase the number of participants, lengthen the timeline of the study and implement a control group. By increasing the sample size of the study, we would be able to draw more concrete and accurate conclusions by analyzing our

results using statistical tests. We would lengthen the timeline of the study by conducting the same methodology on each participant over multiple menstrual cycles, therefore, limiting user errors with the Smart Underwear device and abnormal cycles. This would allow us to compare multiple hormone rates of an individual and gather an average, which could increase the significance of the results.

Future studies could further explore hormonal fluctuations during key phases, such as the peri-ovulatory window (when estrogen peaks sharply before ovulation) or the early-cycle FSH surge (occurring around day five of the menstrual cycle). The estrogen surge represents a hormonal shift that is biologically different from both the menstrual and luteal phases. Meanwhile, the FSH surge plays a critical role in selecting the dominant follicle and facilitating the advancement of the estrogen surge. By tracking GI symptoms, microbial output, and hormone levels during these events, we could identify whether estrogen surges or FSH surges contribute to specific gastrointestinal symptoms or microbiome changes. Including these phases would offer a more comprehensive understanding of how different points in the cycle uniquely affect gut health, and could help identify phase-specific interventions for GI-related disorders in females.

Lastly, implementing a control group would provide baseline hormone levels for comparison throughout the study. Ideally, we could observe participants who have never been on birth control and then introduce them to a hormonal contraceptive that suppresses the natural cycle of hormone production. Having a control group that has a standardized hormone cycle would isolate the GI symptoms and determine the cause of them. Implementing these improvements to our study would yield significantly different results which could be used to inform further studies in the realm of female health research.

Glossary

Accelerometer - Sensor in a device that detect changes in movement and motion (*Accelerometer - an Overview | ScienceDirect Topics*, n.d.).

Anterior pituitary- is a small, pea-sized gland at the base of the brain, just below the hypothalamus. As part of the endocrine system, it regulates multiple hormone-secreting glands—including the ovaries—by releasing key reproductive hormones like luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Cleveland Clinic, 2021).

Bolus - the partially digested food content found in the gut (Patel & Thavamani, 2021).

Dysbiosis - an imbalance of the microorganisms found in the gut (Sidhu & Poorten, 2017).

Endometrium - the mucous membrane lining the uterus (Merriam-Webster, 2024).

Estradiol - A natural hormone that is secreted chiefly by the ovaries, and is the most potent of the naturally occurring estrogens (Merriam-Webster, 2024).

Estriol - A relatively weak natural estrogenic hormone mostly found as a metabolite of estradiol, secreted by the placenta (Merriam-Webster, 2024).

Estrone - A natural estrogenic hormone mostly found as a metabolite of estradiol, secreted by the ovaries (Merriam-Webster, 2024).

Fermentation - the chemical breakdown of a compound by a microorganism that produces gas as a byproduct (Levitt, 1980).

Glycoside hydrolases - enzymes that catalyze the degradation of glycosidic bonds (Withers & Williams, 2022).

Glycosidic bonds - covalent bonds that hold molecules together in order to form carbohydrates and other molecules (Ardèvol & Rovira, 2022).

Glycoprotein hormones - are a class of proteins that are the most complex molecules with hormonal activity. This class of proteins includes three hormones generated from the pituitary gland: gonadotropins follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH) (Cahoreau, 2015).

Gonads - a reproductive gland (such as testes or ovaries) that produces gametes (Merriam-Webster, 2024).

Menopause - the final menstrual period resulting from the physiologic permanent decline in gonadal hormone levels confirmed by 12 months of amenorrhea in females with a uterus (The Academy of Obstetricians and Gynecologists, 2022)

Microbe - a microorganism that occupies a specific habitat and may cause disease (Donaldson et al., 2016).

Microbiome - the combined genetic information of the microorganisms found in one specific location (Sidhu & Poorten, 2017).

Microbiota - a collection of microorganisms that occupy a particular habitat (Sidhu & Poorten, 2017).

Peristalsis - the involuntary process by which food contents in the digestive tract are forced forwards due to the contraction and relaxation of different muscles (Patel & Thavamani, 2021).

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Appendix

Figure A1: Recruitment Flyer

BELI User Experience Consent Form
LH Consent Form.pdf

Figure A2-3: Participant Consent Forms

LH Test Strip User Instructions.pdf

Wearing-Instructions-Smart-Underwear.docx.pdf

Figures A4-5: Case Study Participant Instructions

TEAM BELI STUDY

Participant Identification Number: _____ **Key:** white = wearing the device, grey = NOT wearing the device

Please circle one for these prompts: (Yes/No) or (+ / - / invalid)

Day 1 of menstrual cycle **Date:** _____ WEAR DEVICE I turned the device on at __:__ I wore the device: (Yes/No) I turned the device off at __:__	**Day 2 of menstrual cycle** **Date:** _____ WEAR DEVICE I turned the device on at __:__ I wore the device: (Yes/No) I turned the device off at __:__	**Day 3 of menstrual cycle** **Date:** _____ WEAR DEVICE I turned the device on at __:__ I wore the device: (Yes/No) I turned the device off at __:__	**Day 4 of menstrual cycle** **Date:** _____	**Day 5 of menstrual cycle** **Date:** _____	**Day 6 of menstrual cycle** **Date:** _____	**Day 7 of menstrual cycle** **Date:** _____
Day 8 of menstrual cycle **Date:** _____	**Day 9 of menstrual cycle** **Date:** _____	**Day 10 of menstrual cycle** **Date:** _____	**Day 11 of menstrual cycle** **Date:** _____	**Day 12 of menstrual cycle** **Date:** _____	**Day 13 of menstrual cycle** **Date:** _____ WEAR DEVICE I turned the device on at __:__ I wore the device: (Yes/No) I turned the device off at __:__ I took the LH test (Yes/No) LH test results: (+ / - / invalid)	**Day 14 of menstrual cycle** **Date:** _____ WEAR DEVICE I turned the device on at __:__ I wore the device: (Yes/No) I turned the device off at __:__ I took the LH test (Yes/No) LH test results: (+ / - / invalid)
Day 15 of menstrual cycle **Date:** _____ WEAR DEVICE I turned the device on at __:__ I wore the device: (Yes/No) I turned the device off at __:__ I took the LH test (Yes/No) LH test results: (+ / - / invalid)	**Day 16 of menstrual cycle** **Date:** _____	**Day 17 of menstrual cycle** **Date:** _____	**Day 18 of menstrual cycle** **Date:** _____	**Day 19 of menstrual cycle** **Date:** _____	**Day 20 of menstrual cycle** **Date:** _____	**Day 21 of menstrual cycle** **Date:** _____ WEAR DEVICE I turned the device on at __:__ I wore the device: (Yes/No) I turned the device off at __:__
Day 22 of menstrual cycle **Date:** _____ WEAR DEVICE I turned the device on at __:__ I wore the device: (Yes/No) I turned the device off at __:__	**Day 23 of menstrual cycle** **Date:** _____ WEAR DEVICE I turned the device on at __:__ I wore the device: (Yes/No) I turned the device off at __:__	**Day 24 of menstrual cycle** **Date:** _____	**Day 25 of menstrual cycle** **Date:** _____	**Day 26 of menstrual cycle** **Date:** _____	**Day 27 of menstrual cycle** **Date:** _____	**Day 28 of menstrual cycle** **Date:** _____
Day 29 of menstrual cycle **Date:** _____	**Day 30 of menstrual cycle** **Date:** _____	**Day 31 of menstrual cycle** **Date:** _____				

Figure A6: Participant Smart Underwear Device Wearing Calendar

Day 1	Day 2	Day 3
Meal/Food 1 **Time:** **General Foods Consumed:**	**Meal/Food 1** **Time:** **General Foods Consumed:**	**Meal/Food 1** **Time:** **General Foods Consumed:**
Meal/Food 2 **Time:** **General Foods Consumed:**	**Meal/Food 2** **Time:** **General Foods Consumed:**	**Meal/Food 2** **Time:** **General Foods Consumed:**
Meal/Food 3 **Time:** **General Foods Consumed:**	**Meal/Food 3** **Time:** **General Foods Consumed:**	**Meal/Food 3** **Time:** **General Foods Consumed:**
Meal/Food 4 **Time:** **General Foods Consumed:**	**Meal/Food 4** **Time:** **General Foods Consumed:**	**Meal/Food 4** **Time:** **General Foods Consumed:**
Additional Meal/Food **Time:** **General Foods Consumed:**	**Additional Meal/Food** **Time:** **General Foods Consumed:**	**Additional Meal/Food** **Time:** **General Foods Consumed:**

Figure A7: Participant Food Log

Team BELI User Experience Survey

Having worn the Smart Underwear device for nine days, please answer the following questions.

Please answer the first four questions with:

Strongly agree, somewhat agree, neither agree nor disagree, somewhat disagree, disagree

1. The instructions for using the Smart Underwear device were clear and easy to follow.
2. The Smart Underwear device is comfortable to wear.
3. Wearing the Smart Underwear device didn't disrupt my daily life.
4. The Smart Underwear was securely attached by the double-sided tape and didn't fall off.
5. During the nine-day wearing period, how many days did you wear the Smart Underwear Device?
   - 1
   - 2
   - 3
   - 4
   - 5
   - 6
   - 7
   - 8
   - 9
6. If you stopped wearing the Smart Underwear device, why did you stop?

   Free response or NA.

Having used the LH strips on days 12, 13, and 14 of your menstrual cycle, please answer the following questions.

Please answer the following three questions with: yes or no

1. Did you use a LH strip on Day 12 of your menstrual cycle?
2. Did you use a LH strip on Day 13 of your menstrual cycle?
3. Did you use a LH strip on Day 14 of your menstrual cycle?

Please answer the following question with:

Strongly agree, somewhat agree, neither agree nor disagree, somewhat disagree, disagree

4. The instructions for using the LH strips were clear and easy to follow

Figure A8: Team BELI User Experience Survey