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Associations between gut microbiome metabolomics and endometriosis risk, progression, and non-invasive diagnosis: a review

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Associations Between Gut Microbiome Metabolomics and Endometriosis Risk, Progression, and Non-Invasive Diagnosis: A Review

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Abstract

Endometriosis is a gynecological condition that affects 1 in 10 women of reproductive age, is characterized by growth of endometrial tissue outside of the uterus, and encompasses metabolic, immunologic, and endocrine dysfunction. Despite its significant impact, endometriosis remains inadequately studied within the realm of women's health, emphasizing the crucial need for innovative approaches in the analysis and diagnosis of this complex condition. The cause of endometriosis is unknown however it could be influenced by genetics, environmental factors, diet, lifestyle, and notably composition of the gut microbiome. Recent scholarly interest has sparked inquiries into the correlations between endometriosis and the gut microbiome, and studies have unveiled altered gut microflora profiles with endometriosis as well as identified microbial metabolic byproducts that potentially influence disease development and progression. The associations between endometriosis and the gut microbiome can be evaluated using metabolomics, which is a rising branch in the sciences and medicine that involves the study of metabolites in the body and can be used to identify biomarkers to non-invasively analyze and diagnose a disease. Metabolomic studies have provided evidence for variations in gut microbiota abundance, composition, and diversity among individuals with endometriosis, alongside alterations in metabolites like short-chain fatty acids and estrogen-modulating bacteria that potentially impact the risk and progression of the condition. Leveraging fecal metabolomics to examine distinctions in gut microbiome composition specific to endometriosis, and identifying potential biomarkers, offers a non-invasive avenue for diagnosing the condition. Furthermore, the interplay between dietary factors, the gut microbiome, and endometriosis risk and progression underscore the significance of dietary interventions in mitigating disease outcomes. Evidence supports the beneficial role of fiber intake, omega-3 polyunsaturated fatty acids, foods

rich in phytoestrogens, antioxidants like resveratrol and vitamins C, E, and D, and probiotics in reducing the risk and slowing the progression of endometriosis. While a causal relationship between altered gut microbiota profiles and endometriosis remains elusive, further exploration of the intricate connections between the gut and women's reproductive health is imperative. Studying the associations between endometriosis and the gut microbiome through metabolomics not only holds promise for enhancing diagnostic capabilities, but also for advancing minimally invasive therapeutic strategies in the management of endometriosis and related conditions.

Introduction

Some ongoing research in the field of gynecology has focused on the development of advanced diagnostic methodologies for gynecological conditions using metabolomics (Troisi J et al. 2022). Metabolomics is a rising field of study that involves analyzing metabolites in the body that could be biomarkers or indicators of certain metabolic processes or medical conditions. This field will play an important role in medicine as technology advances considering that it can provide access to non-invasive diagnostic methods.

Further research and application of metabolomics to the domains of female reproductive and gastrointestinal systems is necessary, as there is a notable lack of comprehensive research on the associations between digestive health and reproductive diseases like endometriosis. Given the distinctive hormonal profiles and microbiomes of women, comprehending these intricate correlations is crucial for enhancing diagnostic capabilities, preventive measures, and treatment modalities for disorders affecting the endometrium. Furthermore, female health is lacking sufficient research in general and there is much to discover regarding female reproductive system pathology. Awareness, research, and therapeutic development for conditions like endometriosis

is important to modulate pain and prevent infertility for individuals with such gynecological problems.

Moreover, the complex interplay between the gut microbiome and reproductive health offers a promising avenue for future scientific inquiry. The gut microbiome can exert influence on the host's physiological, psychological, and reproductive traits, while being reciprocally influenced by environmental factors such as diet, immune system function, and exposure to toxins and antibiotics (Talwar et al. 2022). Notably, disruptions in the gut microbiome have been associated with inflammation, compromised epithelial integrity, nutrient absorption issues, and hormonal imbalances, potentially contributing to reproductive tract pathologies, including endometriosis (Talwar et al. 2022; Baker et al. 2017; Nabi et al. 2022; Yuan et al. 2018).

Factors that can influence endometriosis include estrogens, short chain fatty acid production, polyunsaturated fatty acids, vitamins E, C, and D, and probiotics. Endometriosis is an estrogen driven disease, and besides the endometrium, the gut microbiome is a principal regulator of circulating estrogen levels. Additionally, endometriosis is driven by inflammation, and so inflammatory mediators like polyunsaturated fatty acids and antioxidants including vitamins E, C, and D can potentially modulate inflammatory responses and pain. Finally, considering the gut microbiome’s influence on endometriosis progression, promoting a healthy microbial community through probiotic supplementation can be effective.

Advancing the comprehension of the factors influencing endometriosis progression is essential for innovating treatment strategies that alleviate pain and address infertility in affected individuals. This review will address how endometriosis is interconnected with composition and function of the gut microbiome, how using metabolomic studies to evaluate patterns of microbial

biomarkers persistent amongst patients with endometriosis is useful, and how nutrition-based therapeutic approaches can affect endometriosis risk, progression, and pain management.

Materials and Methods

A review of literature was conducted to identify the most relevant studies regarding my topic. I used the UTC library database and especially sources like the National Library of Medicine, Science Direct, and PubMed, and searched for keywords and phrases including "endometriosis," "gut microbiome," "metabolomics," "estrogen-driven disease," "environmental risks for endometriosis," "genetics of endometriosis," "biomarkers of endometriosis," "microbiome dysbiosis," "microbial diversity," "intestinal integrity," "non-invasive diagnostic techniques," "fecal metabolomics," "role of short chain fatty acids," "diet and endometriosis," "nutritional risks of endometriosis," "protective nutritional components against endometriosis," "benefits of a plant based diet for endometriosis," etc. In my review, I established key questions and topics to address.

Results and Discussion

A. What is endometriosis?

Endometriosis is becoming a more familiar and common medical condition across women worldwide, though not long ago and even today still, endometriosis symptoms are passed off as severe menstruation. Individuals are often dismissed for their symptoms, prescribed hormones and birth control that have the potential to make the condition worse and are forced to live with a lifetime of pain. Endometriosis affects one in 10 females of reproductive age, is characterized by growth of endometrial tissue in extra-uterine sites,

and encompasses metabolic-, immunologic, and endocrine disruption (Talwar et al. 2022). In cases of endometriosis, the lining of the uterus, known as the endometrium, grows into the outside of the uterus as well as on other organs of the female reproductive tract including the fallopian tubes and ovaries, and the condition is characterized by lesions of endometrial tissue in these abnormal locations (Kanellopoulos et al. 2022). Endometriosis is one of the most frequently encountered pathological conditions as it affects millions, about 10-15% of females worldwide, half of which (30-50%) present with infertility (Bulletti et al. 2010; Moradi et al. 2021).

During a typical menstruation, the endometrium responds to a drop in estrogen and progesterone levels which causes the endometrium to shed. The shedding of this uterine lining results in menstrual bleeding, cramping, and pelvic pain. For a female with endometriosis where the endometrium has grown outside and around the uterus, the endometrium will respond to hormonal changes in a similar fashion to the interior endometrium. However, because the displaced endometrium has no way to exit the body if it were to shed off the same way, the result is severe inflammation, lesion formation, scarring, and pain (Alimi et al. 2018). Endometriosis can present in four stages: stage 1 (minimal), stage 2 (mild), stage 3 (moderate), and stage 4 (severe), where patients with stage 4 endometriosis often present with lesions beyond the reproductive tract as well as infertility (Smolarz et al. 2021). As imagined, the pain that presents with endometriosis can be debilitating and can impact patients' daily lives. Endometriosis begins its effects during reproductive years and because of its response to hormonal changes, the pain is often more severe during menstruation. Since endometriosis is different for every individual the condition claims, pain can vary from a mild discomfort to extreme pain

that prevents patients from going about their day-to-day activities (Maddern et al. 2020). The clinical presentation of endometriosis can vary greatly from patient to patient, which is one of the reasons why it often goes undiagnosed for years, and why development of new diagnostic techniques is crucial for consistent and accurate diagnosis. Along with the debilitating pain that is common for women with endometriosis, patients with this condition often suffer from emotional and mental distress as well due to the chronic nature of the disease. The physical and emotional struggle that women with endometriosis endure greatly reduces their quality of life, which adds to the reasons why further research in this field is so necessary (Van Stein, 2023).

Endometriosis, along with other conditions within gynecology, is understudied and therefore the cause remains unclear. Though, factors such as genetic predisposition, autoimmune disorders, and retrograde menstruation are believed to contribute to development. Retrograde menstruation is described by the Sampson theory, which proposes that the endometrial tissue typically expelled during a female's period instead flows backward through the fallopian tubes and into the peritoneal cavity, releasing endometrial cells into the ectopic space (Dastur and Tank, 2010). This theory is widely accepted, though is likely not the sole cause of endometriosis onset because while up to 90% of women experience some retrograde menstruation in their lifetime, only 10% of women develop endometriosis, suggesting that other factors contribute to cause (Dastur and Tank, 2010). Recent studies have revealed altered gut microflora profiles during endometriosis and dysregulated microbial metabolic byproducts that potentially impact disease progression, so it is possible that gut microbiome dysbiosis is a contributing factor in endometriosis development (Yuan et al. 2018). Some gut bacteria may

contribute to endometriosis progression while others may provide protection against endometriosis.

Diagnosis is difficult in that there is no way to accurately diagnose endometriosis without painful interventions such as an endometrial biopsy or exploratory surgery, so developing new diagnostic measures will be crucial for the future of endometriosis research. New diagnostic techniques could include performing genome studies to identify genes that may predispose individuals to the disease, metabolic analysis to identify protein biomarkers within host samples, and possibly gut microflora analysis to identify bacteria dysbiosis associated with pathology of endometriosis (Hsu et al. 2010).

Endometriosis management does not include a single action for cure, but instead involves a multidisciplinary approach involving hormonal therapies, pain management, psychological support, surgical interventions, and gastrointestinal treatment to ensure successful remission (Ugwumadu et al. 2017). Because of the gross disposition of endometriosis and its treatments, some patients may have trouble with fertility (Bonavina et al. 2022). Misdiagnosed and mistreated endometriosis cases often lead to premature infertility, which is a reason why developing more efficient diagnostic measures and treatment options is crucial.

B. How is endometriosis an estrogen-driven disease?

Endometriosis is an estrogen-dependent gynecological condition and high estrogen production is a consistently observed endocrine feature of endometriosis (Chantalat, et al. 2020). For many females who have endometriosis, pain during certain durations in the menstrual cycle can be more severe than average. Estradiol, the strongest form of

estrogen responsible for growth and inflammation of endometrial tissue, is mainly produced in endometriotic tissue and local accumulation of this hormone is considered to contribute to endometriosis progression (Chantalat, et al. 2020). Because endometriosis is hormonally driven, hormone supplements are a common treatment method. Hormonal treatments for endometriosis focus on suppressing hormonal fluctuations using gonadotropin and ovarian hormones, causing inhibition of ovulation and menstruation, and resulting in a general decrease in inflammation (Chantalat, et al. 2020). Hormonal treatments can be effective in managing endometriosis symptoms, however these treatments come with significant side effects including vasomotor symptoms, sleep disturbance, urogenital atrophy, bone loss, hot flashes, mood changes, and other inconvenient and negative side effects (Vannuccini, et al. 2022). Because of the potential side effects, it may be necessary to consider alternative, more natural remedies to decrease estrogen levels and promote hormonal balance such as targeting the gut microbiome.

C. Why does endometriosis cluster in families?

The cause of endometriosis is widely unknown and is often random, however because endometriosis can cluster in families, there are likely genetic and/or environmental factors that can impact onset of endometriosis (Dun et al. 2010). Many researchers hypothesize that endometriosis is inherited in a polygenic/multifactorial mode, which is a type of inheritance that occurs when phenotype is determined by a combination of multiple genes and environmental effects (Hansen and Eyster 2010). If a female is exposed to a toxin or toxicant that mutates DNA and genetically disposes them

to endometriosis development, that gene could be passed on to her female offspring; or, if a family lives in an environment where they are all exposed to toxins making them susceptible to induction of endometriosis, the condition can cluster in families. No single gene has been found to be associated with endometriosis, however some consistencies have been observed such as a particular Msp1 polymorphism of CYP1A1 with 95% confidence (Hansen and Eyster 2010). Though, overall, even though many studies have demonstrated familial clustering of endometriosis, the disease does not appear to be inherited in simple Mendelian mode, thus suggesting that other contributing factors such as environment, toxin exposure, and diet/lifestyle are associated with endometriosis familial clustering.

Many studies have proposed and demonstrated that first degree relatives of individuals with endometriosis are 5 to 7 times more likely to have the disease confirmed, suggesting a familial-risk component (Simpson et al. 1980; Kennedy et al. 1997; Ranney B. 1971). Also, some findings have discovered that endometriosis in families tends to be more severe compared to sporadic cases and can also present earlier in life with onset of symptoms at a younger age (Hansen and Eyster 2010). To further study heredity of endometriosis, some twin studies have been conducted which demonstrated higher prevalence of endometriosis in monozygotic twins vs dizygotic twins, which suggests a heritable component (Treloar, S. et al, 1999).

Because of the inconsistency in results and the lack of a definite gene linked with endometriosis, despite familial disease clusters, a Mendelian genetic component is not proven. Other risk factors such as environment, toxin exposure, and diet/lifestyle can have strong association with the development of endometriosis. Environmental exposure

to endocrine disrupting chemicals may be etiologically involved in the onset of endometriosis as well as the severity of the disease (Dutta et al. 2023). Some of the most common endocrine disrupting toxicants we are frequently exposed to in our environment include dioxins, bisphenol A (BPA), phthalates, and dichlorodiphenyltrichloroethane (DDT) (Interdonato et al. 2023). The endocrine system and its regulation have an important relationship to the female reproductive system because the collection of glands that make up the endocrine system produce hormones and release them into the blood. Endometriosis as well as other female reproductive disorders are hormonally driven, primarily by estrogens, and so endocrine disrupting chemicals can interfere with the hormonal homeostasis and can have potential to drive progression of disease (Dutta et al. 2023). Some of the common examples of endocrine disrupting chemicals that we are frequently exposed to are industrial chemical byproducts from plastic manufacturing like bisphenol A and phthalates, as well as dioxins which are byproducts of herbicide manufacturing and use. These chemicals can contaminate soil, water, and crops (Warner et al. 2018). The compound of greatest concern is the dioxin TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin) because of its capacity to be fat soluble, allowing it to be stored within lipid rich foods as well as within our bodies themselves (Gao et al. 2023). Early life exposure to TCDD can simulate a condition that results in chronic inflammation, increasing risk for onset of endometriosis in females (Tanha et al. 2022). TCDD as well as BPAs, phthalates, and other toxicants are all examples of endocrine disrupting chemicals that have been correlated with reproductive disorders including endometriosis (Stephens et al. 2022). In a study conducted in 1993, endometriosis was found to be directly linked to dioxin exposure in monkeys, and the severity of the

condition was determined by the dose given (Rier, et al. 1993). This correlation was likely a result of the dioxin's capability to alter estrogen production in the body.

One of the primary targets of endocrine disrupting chemicals is the endometrium because it responds directly to hormonal fluctuations in a cyclical pattern. Estrogens can help proliferate lesions in the endometrium and excess estrogen is characteristic of endometriosis. Many endocrine disrupting chemicals in the environment affect estradiol production, including BPA which was the first identified toxicant to cause estrogen receptor modification effects (Interdonato et al. 2023). The general public is regularly exposed to BPA because it is a common industrial chemical used to make plastics that can contaminate food, water, air, soil, and has hormone-like properties allowing it to agonize a myriad of estrogen receptors in the body. Endometriosis is directly linked to high levels of BPA and other similar toxicants in the body (Dutta et al. 2023; Interdonato et al. 2023).

Along with the general correlation between endocrine disrupting chemicals and endometriosis development, studies have examined the relationship between endocrine disrupting chemicals and alterations in the gut microbiome, suggesting an additional indirect relationship between environmental toxins and endometriosis. The hormonal alterations that result from endocrine-disrupting chemical exposure can modify the gut microbiota and cause dysbiosis; toxicant-induced gut dysbiosis sensitizes the intestinal barrier and depletes healthy microorganisms that maintain the gut integrity, dispositioning the gut to inflammation that can be correlated with endometrial lesion progression (Fabozzi et al. 2022). For example, BPA exposure has been reported to change the microbial population in the colon and chronic exposure of women to BPA was

found to produce pro-inflammatory gut microbiota with reductions in stress regulating species (López-Moreno et al. 2021). Particular microorganisms in the gut function to reduce oxidative stress and limit absorption and effect of toxins, and so maintaining a healthy diet to promote a healthy gut microbiome can prevent endocrine disrupting chemical induced endometriosis (Kunst et al. 2023).

A variety of exposure pathways that may elevate or lower estrogen levels should be considered to explain familial clustering of endometriosis. The majority of endocrine disrupting chemical exposure occurs via diet, and so maintaining a healthy diet rich in organic foods while avoiding processed foods can help minimize exposure, considering that organic farming systems avoid the use of fertilizers produced from industrial waste, thus avoiding excess toxicant contamination (Peivasteh-Roudsari et al. 2023; Rahman et al. 2024). Evidence supports the benefits of consuming fresh foods, avoiding food in plastic packaging, and avoiding canned food and beverages to reduce dietary exposure to endocrine disruptors that have potential to cause estrogen receptor modification effects (Corbett et al. 2022).

Evaluating the relationships between the environment and family clustering of endometriosis while noting that endometriosis is not directly inherited in a Mendelian manner prompted the question of whether endometriosis correlated with gut microbiome dysbiosis can be passed from mother to child. No evidence for this specific inquiry could be found nor ongoing studies of a heritable relationship between gut microbiome dysbiosis and endometriosis, though this may be an important research question to address as it could potentially affect the recommended diet proposed during pregnancy.

D. What is metabolomics and how does it apply to the gut microbiome?

Metabolomics is a rising branch in the sciences and medicine that involves the study of metabolites in the body. Metabolites are molecules that exist within cells, tissues, and biological fluid and are a product of cellular processes. Metabolite levels and certain metabolite prevalence can provide real-time insight into how the body is functioning physiologically as well as the biochemical balance of the body. Certain metabolites could be biomarkers or indicators of certain metabolic processes or medical conditions; therefore, identifying and quantifying important metabolites can offer a snapshot of the biochemical reactions and metabolic pathways within an organism (Khoo and Al-Rubeai, 2007). Along with a deep understanding of basic cellular function, we can use this tool to diagnose conditions such as endometriosis as well as evaluate how disposition and external factors affect homeostasis within the body. Metabolomics is the next step toward personalized medicine and non-invasive diagnostics and will play an important role in the medical field as technology advances.

Metabolomics can play a significant role in gut microbiome research of metabolites produced by the microorganisms that make up the gut microbiome. In recent years and for many years to come, the gut microbiome has been a topic of interest because of its correlations to myriad systems of the body. Numerous studies have been conducted on the gut-brain association and new correlations of interest are beginning to emerge. Metabolomics can help researchers identify and quantify metabolites produced by gut bacteria, understand how these metabolites are impacted by the host's diet, characterize metabolites by distinguishing bioactive compounds, identify specific biomarkers for dysbiosis of microbial activity, provide insight into the correlations between metabolism

and the gut microbiome, and contribute to our understanding of how someone's unique microbial community impacts their health and predisposition to conditions such as endometriosis (Bauermeister et al. 2021). Overall, characterizing and analyzing the gut microbiome will be crucial to understanding human disease on a new level.

Through recent research, metabolomics has shown to be a useful tool in identifying biomarkers and significant metabolites associated with endometriosis. Using metabolomics to identify biomarkers for endometriosis could involve sampling biofluids such as blood, saliva, urine, or feces. Delay in diagnosis and misdiagnosis is one of the biggest challenges in endometriosis treatment because without efficient diagnostic techniques, endometriosis is often confirmed after the condition has already progressed, increasing the risk of infertility. In patients with inconsistent or atypical symptoms, diagnosis could be delayed for as long as 10 years (Hadfield, et al. 1996). Development of more efficient diagnostic measures could be groundbreaking in achieving early diagnosis and treatment for the brutal disease as well as to prevent infertility. Diagnosis using metabolomics is safe and non-invasive and considering that presentation of endometriosis often varies from patient to patient, metabolomics can help ensure early diagnosis more consistently.

Metabolomics begins using technology such as NMR and mass spectrometry to create a metabolic profile from a biological sample, and even though both techniques have disadvantages that could compromise the accuracy of results, using both NMR and mass spectrometry together can provide a complete picture of the metabolome (Ortiz, et al. 2021). The process of generating a metabolomic profile involves first collecting samples, preparing them for extraction, employing analysis techniques such as NMR and

mass spectrometry, and then statistically analyzing the presence and quantity of metabolites through methods such as principal component analysis (PCA) to assess metabolome distinctions between experimental and control groups (Wishart, et al. 2018; Ortiz, et al. 2021; Yang, et al. 2019). Once metabolomic profiles are generated and disease biomarkers are identified, metabolomics can identify disease biomarkers and can possibly be used to monitor disease progression as well, as demonstrated by a preliminary metabolomic study on endometrial cancer (Troisi et al. 2022). Metabolomic studies of endometriosis have been conducted to analyze endometrial tissue serum, follicular fluid, urine, and endometrial fluid (Ortiz et al. 2021); however, few metabolomic studies of endometriosis have been conducted to analyze fecal samples to identify biomarkers within the gut microbiome, which could be significant considering rising knowledge of the associations between the gut microbiome and the female reproductive tract.

E. Overview of the Gut Microbiome and Intestinal Dysfunction

Gut microbiome composition varies from person to person, and a healthy balance of microorganisms for one individual may differ entirely from that of a different individual. A stable and healthy microbiome depends on lifestyle, exercise frequency, and dietary habits (Rinninella et al. 2019). So, there can be no indication for a specific, optimal gut microbiome composition. Typically, the millions of microorganisms in our gut make up about six different phyla including Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusonacteria, and Verrucomicrobia, as represented in figure 1, with the two phyla Firmicutes and Bacteroidetes representing 90% of gut microbiota (Rinninella et al. 2019). The Firmicutes are largely represented by genera including *Clostridium*,

Lactobacillus, Bacillus, Enterococcus, and Ruminococcus. Bacteroidetes are largely represented by *Bacteroides* and *Prevotella* (Arumugam, et al., 2014).

Figure 1:

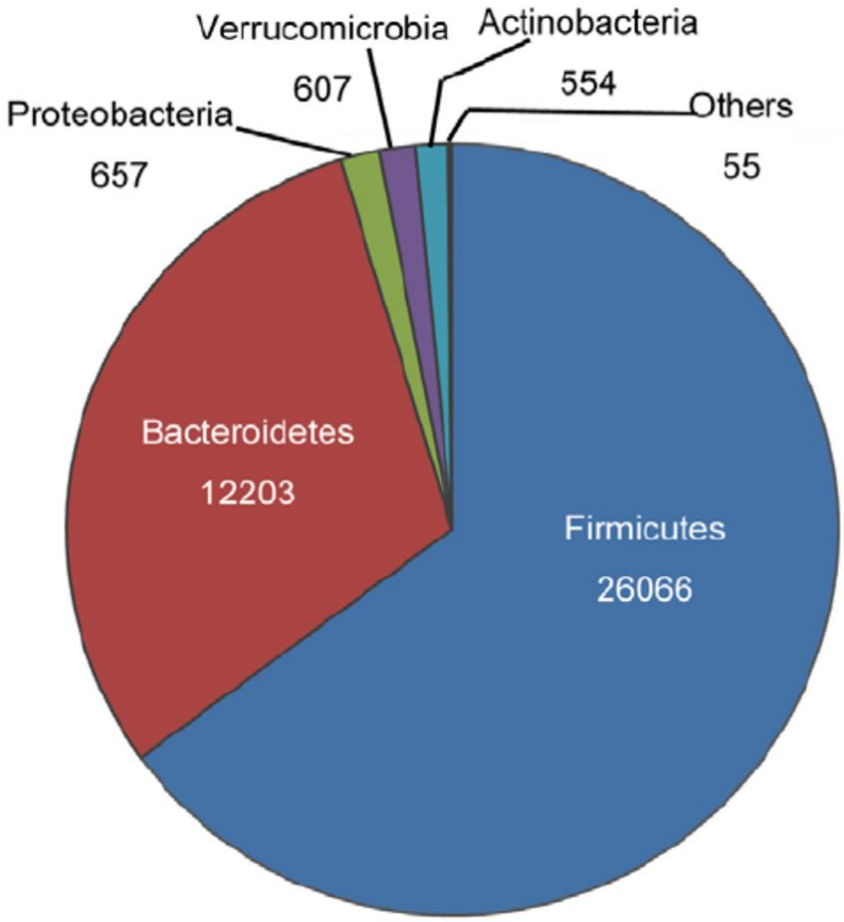


Figure 1 depicts relative abundance ratios of the six major phyla that make up the gut microbiome, as described by Yang et al. 2009.

Considering that certain bacteria in the gut have specific roles in regulating the immune system and the inflammatory response, overrepresentation or under-representation of certain bacterial species can influence the production of inflammatory

molecules. For instance, a healthy gut typically has high representation of Firmicutes and Bacteroidetes which are phyla that induce regulatory T cells that modulate Th17 cells and cytokine production, which are responsible for intestinal inflammation (Kosiewicz et al. 2011).

The bacteria in our gut play important roles in metabolism and basic physiological processes because of the catalytic pathways performed by the microbes (Rowland et al. 2018). These microbes aid in regulating host metabolism, hormones, inflammatory responses, insulin sensitivity, glucose tolerance, fat storage, appetite, and maintaining intestinal epithelium integrity (Talwar et al. 2022; Camillerie et al. 2019). Some of the most important metabolic products of the gut microbiome include anti-inflammatory macromolecules and short chain fatty acids (SCFAs) which are both associated with the gut-reproductive tract axis through regulating intestinal integrity, immunity, and hormone production (Ashwitha et al. 2024).

F. How is gut microbiome dysbiosis associated with disease outside the gastrointestinal tract?

Dysbiosis of the gut microbiome refers to an imbalance of the composition of the microbial community that lives within the gut, and often leads to functional issues and can promote disease states (Messer and Change, 2018). The functional issues that result from gut microbiome dysbiosis don’t just affect our digestion but can also affect homeostasis throughout the body. Dysbiosis results from a shift in the relative abundance or diversity of microorganisms in the gut and is most influenced by genetics, diet, age, antibiotics, infection, stress, or inflammatory conditions such as irritable bowel syndrome

(Shaikh et al. 2023). When the microbiome is imbalanced, the way we absorb nutrients, digest food, protect ourselves from pathogens, and regulate our immune system can become disrupted, and so chronic gut microbiome dysbiosis can influence a variety of health conditions, even endometriosis (Hrncir et al. 2022). Once knowledge of the essential gut bacteria and how they can become imbalanced is gained, an understanding of how the gut microbiome is linked to the rest of the body and how dysbiosis of our gut can influence inflammatory conditions like endometriosis can follow.

The gut microbiome can be directly linked to inflammation because the balance of bacteria in our gut influences our body's defense system including immunity and regulation of oxidative stress (Yoo et al. 2020). Additionally, the gut bacteria play important roles in maintaining intestinal integrity through protection of the epithelial layer. A dysbiotic microbiota can compromise the intestinal barrier, which can lead to exposure of molecules from the diet and microbiome to surrounding tissues and organs and can further impact the host immune system and metabolism (Hrncir et al. 2022), while a healthy gut microbiome can maintain the intestinal barrier by supplying energy for the intestinal epithelial lining and helping to regulate the tight junctions between epithelial cells to prevent leakage (Paradis et al. 2021). A compromised barrier could result in what's known as leaky gut syndrome. Leaky gut is a syndrome where the intestinal integrity is compromised and increased permeability can occur. The layer of epithelial cells protecting the intestinal wall plays an important role in the immune system and metabolic function because this barrier maintains necessary nutrients for digestion as well as bacteria in the confined space (Camillerie et al. 2019). When harmful substances that are normally contained within the gut escape into the bloodstream and the

body recognizes the bacteria and waste as foreign, an immune response can trigger (Hrncir et al. 2022). Without intervention, leaky gut syndrome can cause chronic inflammation and dysregulation of the immune system, which can contribute to a variety of health conditions, including endometriosis (Talwar et al. 2022).

Along with leaky gut, there may be a correlation between endometriosis and inflammatory bowel disease (IBD). Inflammatory bowel disease is a chronic inflammatory condition characterized by inflammation along the digestive tract. The two primary conditions that make up inflammatory bowel disease include Crohn's disease and ulcerative colitis, both of which involve some degree of inflammation along the digestive tract, most centralized to the gut. Studies have shown that some endometriotic women co-develop inflammatory auto-immune disorders such as IBD because both endometriosis and IBD are influenced by gut microbiome dysbiosis with a loss of bacterial diversity and an imbalanced ratio of Firmicutes/Bacteroidetes (Elinav et al. 2011). According to a meta-analysis conducted in 2022, women with endometriosis are three times more likely to have some form of irritable bowel syndrome compared to women without endometriosis (Nabi et al. 2022). While IBD can often co-develop with endometriosis, a leaky gut on the other hand is more likely to contribute to endometriosis disease progression.

G. How is dysbiosis of the gut associated with endometriosis specifically?

Research is emerging regarding the connections between endometriosis and dysbiosis of the gut microbiome for several reasons: dysbiosis may contribute to the inflammation response associated with endometriosis, dysbiosis of the gut may

negatively affect the immune system through altered production of short-chain fatty acids, dysbiosis may cause an increase in intestinal permeability leading to displaced bacteria and greater inflammation, and dysbiosis has the potential to alter estrogen metabolism and an imbalance in estrogen levels can affect endometriosis progression (Qi et al. 2021; Xholli et al. 2023) . The two main drivers of endometriosis progression are immune and hormonal dysregulation, both of which are profoundly governed by the microbial members of the gut (Talwar et al. 2022). Because, however, there are several correlations between gut microbiome dysbiosis and endometriosis with little evidence to establish causal relationships, and because it is unclear whether altered gut microbiota profiles are a result of endometriosis or a contributing cause, further research is necessary.

Some early research on the correlations between endometriosis and gut microbiota studied how induction of endometriosis in mice altered the gut microbial flora profile, and after 21 days and 42 days of endometriosis progression, a general decrease in microbial diversity was observed (Ni, et al, 2020). That project sequenced the microbiome of mice induced with endometriosis to evaluate gut microbiota alterations. Researchers describe a metabolome of 156 metabolites and describe a general decrease in microbial diversity and abundance. More specifically, the researchers recognized an elevated ratio of Firmicutes/Bacteroidetes as well as increased abundance of Acidobacteria, Actinobacteria, Cyanobacteria, Fusobacteria, Patescibacteria, Proteobacteria, and Saccharbacteria in the mice.

A study conducted in 2019 compared gut microbiota composition between women with stage 3/4 endometriosis and healthy controls, which revealed significant differences

in genera and species abundance (Ata et al. 2019). An elevated ratio of Firmicutes/Bacteroidetes is exemplified in Figure 1, where the species *Bacteroides, Prevotella, and Parabacteroides* belonging to phylum Bacteroidetes were less abundant in gut microbiomes of endometriosis patients, and the species *Faecalibacterium, Roseburia, Blautia, and Coprococcus* belonging to the phylum Firmicutes were more abundant. Figure 2 shows a significantly different abundance of *Barnesiella* bacteria, which belong to the phylum Bacteroidetes, between stage 3-4 endometriosis and healthy controls.

Figure 2:

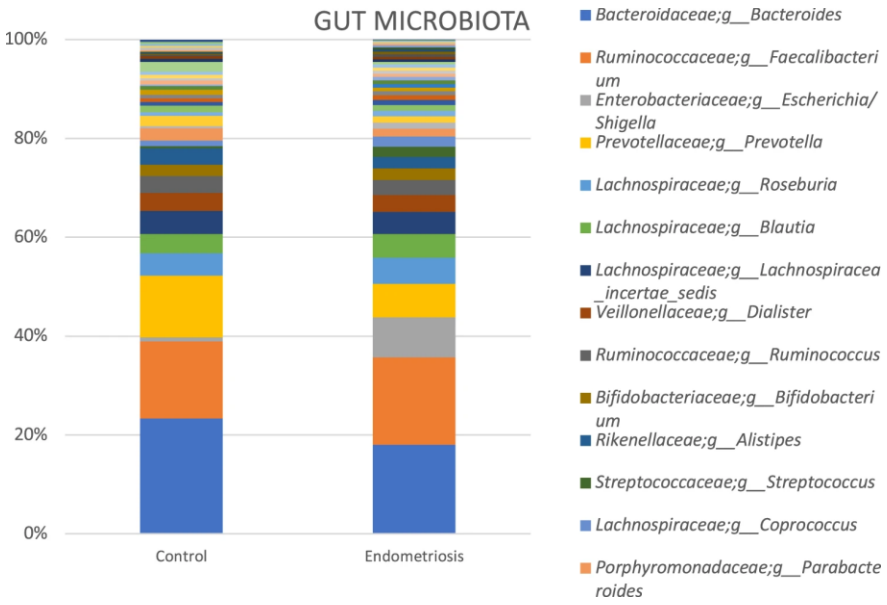


Figure 2 depicts results from a study conducted by Ata et al. in 2019 comparing gut microbiota abundance in fecal samples between endometriosis patients and healthy controls.

Figure 3:

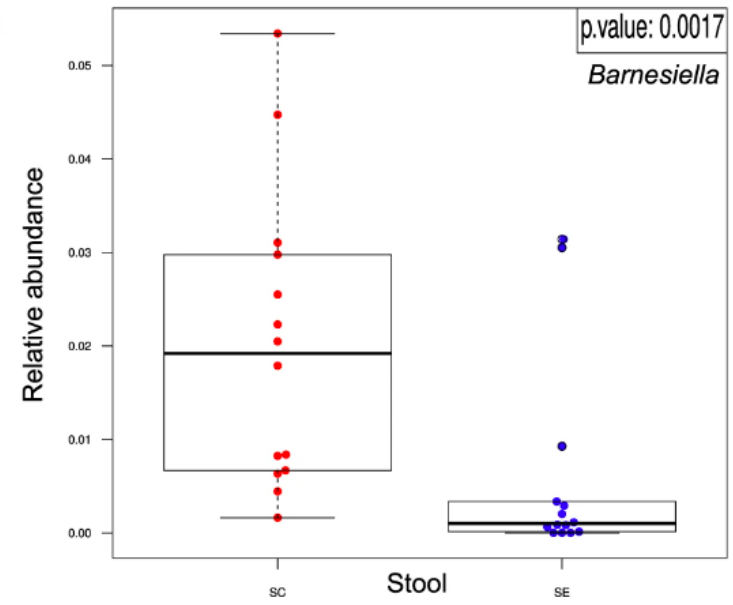


Figure 3 depicts differences in abundance of *Barnesiella* species, belonging to the phylum Bacteroidetes, in fecal samples of endometriosis patients (right) and healthy controls (left).

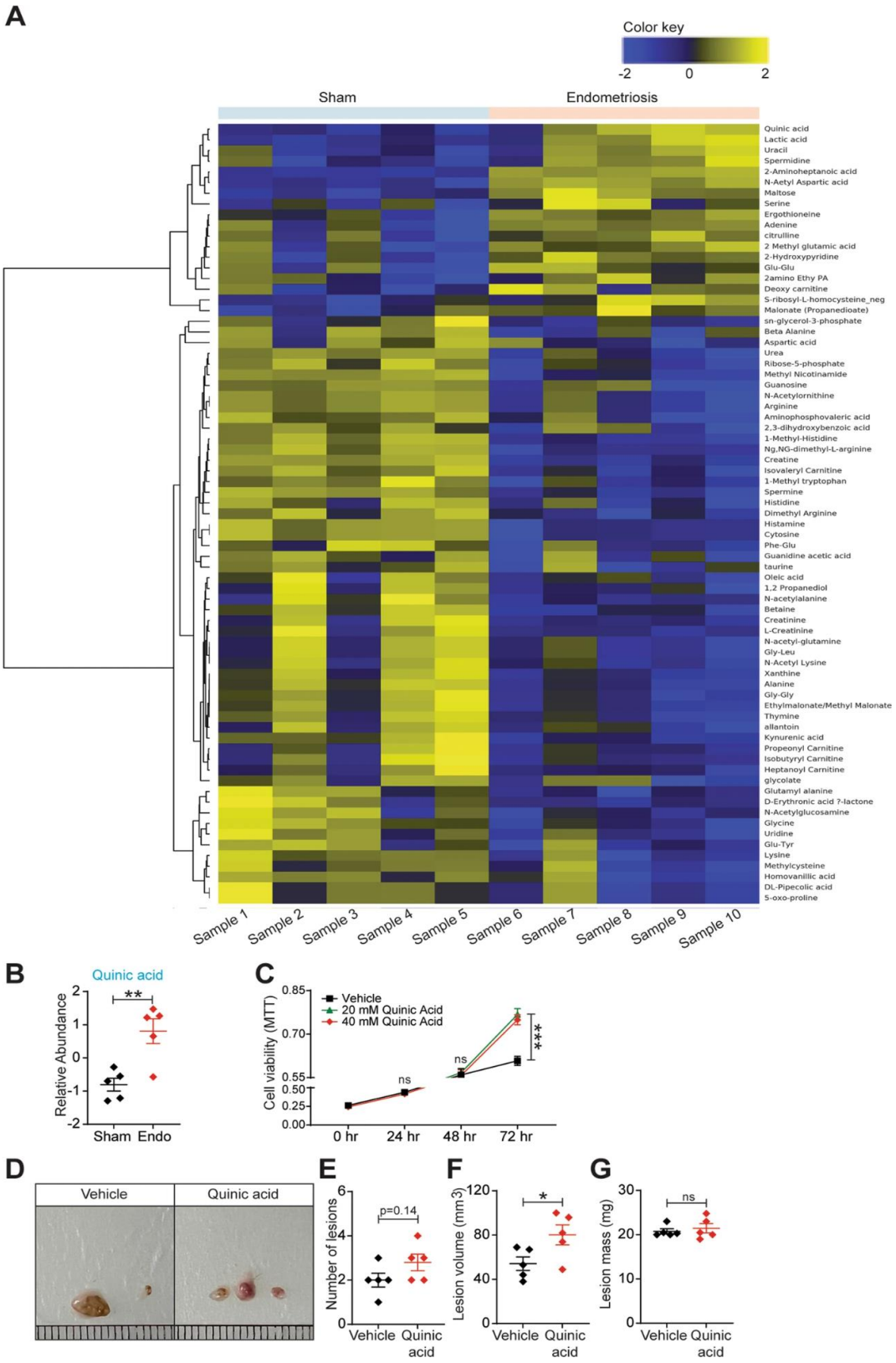
Elevated ratios and abundance of certain bacteria including Acidobacteria, Actinobacteria, Cyanobacteria, Fusobacteria, Patescibacteria, Proteobacteria, and Saccharbacteria observed in mice, can contribute to endometriosis progression because of their proinflammatory potential. High presence of these species of bacteria was found to be correlated with high numbers of macrophages in mice (Chadchan et al. 2021). Furthermore, gut microbial imbalances can alter metabolite production, specifically production of short chain fatty acids. Short chain fatty acids are produced in the gut as a result of fermenting dietary fiber and have significant influence on the integrity of the gut

and on regulation of immune responses (Xiong et al. 2022). The same study that evaluated macrophage presence in mice evaluated concentrations of short chain fatty acids and found that concentration was reduced in endometriosis-induced mice (Chadchan et al. 2021). Following this discovery, the researchers supplemented some endometriosis induced mice with dietary short chain fatty acids and observed some reduction in endometriotic lesions. Short chain fatty acids demonstrate protective action against inflammation and auto-immune responses through inhibiting histone deacetylases which contribute to inflammation via the activation of monocytes and macrophages (Zhao et al. 2019). The two primary mechanisms through which short chain fatty acids such as n-butyrate impact the gut are activation of G-protein-coupled receptors, which are known to downregulate inflammation (Bhatt et al. 2018), and inhibition of histone deacetylases. Researchers have tested the effects of n-butyrate-mediated inhibition on histone deacetylase activity and observed suppressed growth of endometrial lesions, implying that microbial imbalance in which short chain fatty acid production is reduced can reduce protective measures against lesion growth. N-butyrate was shown to inhibit growth of human endometriotic cells both in vitro and in vivo in a preclinical mouse model (Chadchan et al. 2021). Further research is necessary; however, these studies provide supporting data for potential causality between gut microbiome dysbiosis and endometriosis progression in that some bacteria in the gut can promote endometriosis through macrophage-mediated inflammation while others protect against endometriosis by fermenting fiber to produce short chain fatty acids.

An additional study conducted by Sangappa Chadchan, two years after the initial discovery of the relationship between microbial abundance, short chain fatty acid

production, and endometriosis lesions, compared more specific metabolites in fecal samples between endometriosis induced mice and control mice (Chadchan, 2023). The results of this study revealed several signature metabolites, the most significantly distinct metabolites including Quinic acid, Cytosine, 1-Methyl-Histidine, Ng, NG-Dimethyl L-Arginine, 2-Aminoheptanoic acid, and N-Acetyl Aspartic Acid, as represented in figure 4. The effects of these metabolites on endometriotic lesion growth were tested in vitro on cells derived from human endometriosis lesions which revealed that Quinic acid had the most significant effect on lesion proliferation of endometrial epithelial cells, while the other metabolites only moderately affected proliferation. A further study was then conducted on Quinic acid (QA) by orally gavaging endometriosis induced mice with QA every 24 hours for 2 weeks, which revealed that mice who were administered the QA developed significantly larger lesions than the control group of endometriosis induced mice.

Figure 4:


A
Color key
-2
0
2
Sham
Endometriosis
Quinic acid
Lactic acid
Uracil
Spermidine
2-Aminoheptanoic acid
N-Aetyl Aspartic acid
Maltose
Serine
Ergothioneine
Adenine
citrulline
2 Methyl glutamic acid
2-Hydroxypyridine
Glu-Glu
2amino Ethy PA
Deoxy carnitine
S-ribosyl-L-homocysteine_neg
Malonate (Propanedioate)
sn-glycerol-3-phosphate
Beta Alanine
Aspartic acid
Urea
Ribose-5-phosphate
Methyl Nicotinamide
Guanosine
N-Acetylornithine
Arginine
Aminophosphovaleric acid
2,3-dihydroxybenzoic acid
1-Methyl-Histidine
Ng,NG-dimethyl-L-arginine
Creatine
Isovaleryl Carnitine
1-Methyl tryptophan
Spermine
Histidine
Dimethyl Arginine
Histamine
Cytosine
Phe-Glu
Guanidine acetic acid
taurine
Oleic acid
1,2 Propanediol
N-acetylalanine
Betaine
Creatinine
L-Creatinine
N-acetyl-glutamine
Gly-Leu
N-Acetyl Lysine
Xanthine
Alanine
Gly-Gly
Ethylmalonate/Methyl Malonate
Thymine
allantoin
Kynurenic acid
Propeonyl Carnitine
Isobutyryl Carnitine
Heptanoyl Carnitine
glycolate
Glutamyl alanine
D-Erythronic acid ?-lactone
N-Acetylglucosamine
Glycine
Uridine
Glu-Tyr
Lysine
Methylcysteine
Homovanillic acid
DL-Pipecolic acid
5-oxo-proline
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Sample 7
Sample 8
Sample 9
Sample 10
B
Quinic acid
Relative Abundance
**
Sham
Endo
C
Vehicle
20 mM Quinic Acid
40 mM Quinic Acid
Cell viability (MTT)
ns
ns
***
0 hr
24 hr
48 hr
72 hr
D
Vehicle
Quinic acid
E
Number of lesions
p=0.14
Vehicle
Quinic acid
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Lesion volume (mm3)
*
Vehicle
Quinic acid
G
Lesion mass (mg)
ns
Vehicle
Quinic acid

Figure 4 demonstrates the results of an experiment conducted by Chadchan et al. 2023 depicting signature gut microbiota metabolic products compared between endometriosis induced mice and control mice. Each row in the heat map is a metabolite while each column is a sample collected from an individual mouse (A). Some metabolites including Quinic acid, Cytosine, 1-Methyl-Histidine, Ng, NG-Dimethyl L-Arginine, 2-Aminoheptanoic acid, and N-Acetyl Aspartic Acid were significantly more abundant in endometriosis feces which could be indicative of particular microbiome-derived metabolites associated with endometriosis progression. Quinic acid was significantly present at higher levels in feces of mice with endometriosis and so further assays were conducted on this metabolite (B, C). Investigators further studied Quinic acid's effects on number of lesions, lesion volume, and lesion mass (D, E, F, G).

Considering that endometriosis is an estrogen-driven disease and upregulated estrogen levels can contribute to lesion growth, it is important to evaluate where estrogen is produced and regulated in the body. Besides the endometrium, the gut microbiome is one of the principal regulators of estrogen levels in the body, as the microbiota is involved in the regulation of estrogen cycling (Qi et al. 2021). The gut microbiota regulates estrogen through secretion of beta-glucuronidase which is an enzyme that metabolizes estrogens from their conjugate forms to their deconjugated forms, enabling estrogens to bind to receptors (Baker et al. 2017; Qi et al. 2021). Gut microbiome dysbiosis resulting in increased abundance of beta-glucuronidase-producing bacteria can elevate levels of circulating estrogens and drive endometriosis progression. The gut microbiome in patients with endometriosis may have an increased number of beta-

glucuronidase producing bacteria contributing to disease progression, and so mediating balance of the gut microbiota will promote hormonal regulation and homeostasis (Arnone et al. 2023). According to a study in Poland, higher levels of beta-glucuronidase have been associated with higher circulating estrogens and lower fecal excretion of estrogens in premenopausal women (Mroczyńska and Libudzisz, 2010). Beta-glucuronidase enzymes are responsible for regulating estrogen cycling, and if levels of these enzymes are too high, estrogens can be reabsorbed rather than eliminated, leading to increased levels of estrogens in the body (Qi et al. 2021). Beta-glucuronidase is produced by the intestinal epithelium and by certain intestinal bacteria including *Escherichia coli*, Bacteroides species, and *Clostridium perfringens* (Skar et al. 1988). Factors that can increase beta-glucuronidase levels include antibiotics, dietary red meat, processed foods, sugar, and alcohol (Walaszek, 1990). Establishing a healthy gut microbiome and eating foods high in glucuronic acid including apples, Brussel sprouts, broccoli, cabbage, and oranges can potentially help modulate levels of beta-glucuronidase in the intestines, considering that the liver is working well to perform phase 2 detoxification (Walaszek 1990). Beta glucuronidase inhibitors could quantitatively affect hormone disposition but results of a study on these inhibitors did not show that they significantly reduce risk in breast cancer, which is another estrogen driven disease (Gao et al. 2022; Heerdt et al. 1995). This suggests that estrobolome disposition is a multidimensional process and other factors could contribute.

Considering the possible relationships between endometriosis and gut microbiome dysbiosis including altered bacterial ratios, decreased microbial diversity, decreased production of protective metabolites like short chain fatty acids, and an upregulated

production of estrogen which can drive the disease, further research is necessary to establish causal relationships so that availability of non-invasive treatment options expands. There is no doubt, however, that there are correlations between the gut microbiome and the female reproductive tract, and their relationship should be considered in treatment for endometriosis.

H. How can we use gut microbiota profiling to diagnose endometriosis in a non-invasive manner?

Through metabolomic techniques, we can characterize and analyze the composition of the gut microbiome to identify dysbiosis patterns, identify specific bacteria that may indicate presence of a condition, and identify metabolic products that have association with a condition. Dysbiosis patterns can be analyzed through sequencing metabolite profiles to identify biomarkers and microbial signatures. If researchers can identify a specific pattern of biomarkers indicating a microbial composition persistent amongst patients with endometriosis, we can use these sequencing methods to noninvasively diagnose endometriosis.

Fecal metabolomics and stool screening tests have been commonly used for decades to diagnose and monitor inflammatory bowel syndrome, to screen for colon cancer using DNA stool screening, and to assess fatty acid content and liver function (Karu et al. 2018). Sequencing techniques can be used to identify specific bacteria and metabolites in the gut that are especially abundant in females with endometriosis as compared to healthy females. For example, altered levels of Proteobacteria, Firmicutes, and Bacteroidetes have been found in endometriosis patients (Ni, et al, 2020). Considering the recent

discoveries of the relationships between the gut microbiome and reproductive diseases such as endometriosis, it can be hypothesized that fecal metabolomics can be used to indirectly diagnose endometriosis as a noninvasive alternative to surgical diagnosis.

A study on fecal metabolomics in 2018 proposes several recommendations for fecal metabolomic testing including standardization of collection and sample storage (Karu et al. 2018). These researchers also recommend using more than one type of instrumental analysis technique, such as using NMR along with different forms of mass spectrometry, to gain coverage of the whole metabolome. Using fecal metabolomics will be advantageous to the future of diagnostic medicine because of its non-invasive sampling nature, broad metabolic coverage reflecting many different biological processes, and the characterization of the gut microbiome and how its activity relates to other physiological processes in the body (Karu et al. 2018). A study conducted in 2020 specifically investigated characteristics of fecal metabolomics associated with endometriosis using endometriosis-induced mice. Through screening over 100 different metabolites in endometriosis-induced mice and a control group, the researchers identified decreased abundance in several metabolites within the experimental group and abnormalities suggesting dysbiosis of gut bacteria, thus suggesting that fecal metabolomics could be a tool to analyze and potentially diagnose the disease in the future (Ni et al. 2020).

Although, because fecal metabolomic testing may be prone to variation, more susceptible to cohort effects, and interindividual variability depending on diet, bowel activity, etc., this method may not reveal a clear metabolic trend or be reliable to identify biomarkers consistently. Even so, conducting stool screenings for individuals with reproductive conditions such as endometriosis could prove advantageous in detecting gut

microbiome imbalances (such as altered levels of n-butyrate and beta-glucuronidase) linked to the condition. Subsequently modifying dietary habits or incorporating supplements based on these findings may aid in symptom relief and decelerating lesion progression. Fecal metabolomic studies have been able to show alterations in fecal amino acids, monosaccharides, and short chain fatty acids (Karu et al. 2018), and imbalances in these metabolites can be linked with altered gut bacteria activity and resulting inflammation. Further research and technological developments in the realm of fecal metabolomics will lead to establishment of a new, non-invasive approach to analyzing diseases and how the gut microbiome is associated with disease inside and outside the gastrointestinal tract.

I. How can a direct microbiome-based treatment for endometriosis be effective?

Fecal microbiota transplantation may be an effective treatment for endometriosis patients who have a severely dysregulated microbiome, considering that gut bacteria play a major role in endometriosis progression and lesion formation. During this procedure, feces from a healthy subject, preferably from a person who shares a similar diet and everyday lifestyle, are collected from the donor, and introduced in the patient's digestive tract. Before transplantation, a donor sample is carefully screened for pathogens and bacterial balance. In the 2021 study conducted by Chadcan et al., the researchers transplanted feces from healthy mice into mice with induced endometriosis, and results showed reduced lesion growth when compared to endometriotic mice transplanted with feces from other endometriotic mice. However, because endometriosis is also influenced

by other factors, a fecal transplant would likely not be a cure, rather than an effort to reduce inflammation and protect the intestinal epithelium from further damage.

J. How does diet and nutrition affect endometriosis risk, progression, and pain management?

A change in diet can be an effective and sustainable action to control symptoms of endometriosis and prevent progression of endometriotic lesions. Currently, many medical professionals recommend hormonal therapies and/or surgery as a first line of treatment, which are invasive options, and likely an unfortunate byproduct of the current lack of diagnostic techniques to catch endometriosis early. For individuals with severe symptoms, surgery can provide some temporary relief, however symptoms recur in up to 75% of patients within two years (Chantalat et al. 2020). If new non-invasive diagnostic techniques allow endometriosis to be caught in its early stages, a nutrition-based treatment approach can improve the state of the gut microbiome which can help to decrease endometriosis related inflammation and prevent disease progression. Some examples of dietary components that can positively impact endometriosis include fiber intake to promote short chain fatty acid production, foods containing phytoestrogens, polyunsaturated fatty acids including omega-3 compounds, foods rich in antioxidants, and probiotic supplements.

Studies have shown that certain dietary components can directly affect hormone production and regulation. For example, dietary phytoestrogens are bioactive compounds found in legumes and soy that have estrogenic activity and may help regulate estrogen levels through competing with estrogen for binding sites (López et al. 2020; Xu et al.

1998). Phytoestrogens display estrogenic activity through their structural similarity to estrogens and may help to modulate the endocrine system through competing with estrogens for binding sites, but due to a variety of factors such as exposure, ethnicity, and current hormonal status, effects of dietary phytoestrogens may vary and should not be relied solely for hormonal treatment. Even so, efforts through increasing consumption of phytoestrogen rich foods including soy, fruits, and vegetables could have positive results. Some studies have observed significant decrease in estrogen levels in premenopausal women after the consumption of soy products. A randomized controlled cross-over trial involving 12 premenopausal women resulted in lower urinary concentrations of total estrogens and other inflammatory metabolites for women who consumed a high-soy diet for three menstrual cycles compared to individuals on a low-soy diet (Xu et al. 1998). In Addition to soy, the antioxidant resveratrol can also have anti-proliferative effects against endometriosis. Resveratrol is a compound commonly found in grapes and red wine and functions as a phytoestrogen, similar to soy, and so this compound can help regulate estrogen levels in the body which can control endometriosis progression (Sinha et al. 2017). A study conducted on mice showed that high levels of supplemented resveratrol reduced proliferation of grafts of human endometrium in mice (Amaya et al. 2015). These studies provide evidence that consuming phytoestrogens can aid in decreasing estrogen levels in the body and in turn have a protective role against reproductive diseases like endometriosis, however a causal relationship cannot be determined because physiology and homeostasis vary across individuals.

Studies have also shown that a high intake of polyunsaturated fatty acids, specifically omega-3 fatty acids, can help reduce inflammation in the body because they are anti-

inflammatory mediators (Barnard, et al. 2023). Reducing inflammation in the body could potentially help alleviate symptoms of endometriosis, since the condition is driven by inflammation, and may potentially improve fertility outcomes. In a study conducted in 2013, investigators hypothesized that omega-3 polyunsaturated fatty acids exhibit a protective action against inflammation for endometriosis patients, and through using two groups of endometriosis-induced mice, they determined using lipidomic analysis that omega-3s are a key metabolite in inflammation suppression (Tomio et al. 2013). Both groups of endometriosis-induced mice were given supplements of omega-6 fatty acids while one group was able to convert omega-6 to omega-3 more effectively than the other group, allowing the investigators to evaluate the effect of omega-3 fatty acids on endometriosis lesion growth. Results of the experiment showed that in mice with increased omega-3 levels, lesion development was reduced to fewer than half that of wildtype mice, suggesting that increased omega-3 polyunsaturated fatty acids are associated with the suppression of endometriosis (Tomio et al. 2013; Covens, et al. 1988). The ability of omega-3 fatty acids to suppress inflammation and lesion growth in endometriosis patients could be due to their effect on the gut microbiome. Omega-3s affect the gut microbiome through modulating the type and abundance of gut microbes and through regulating levels of short chain fatty acids which have shown to modulate inflammation and promote intestinal integrity (Fu et al. 2021). Considering the importance of gut microbiome diversity, production of short chain fatty acids by microbes, and overall intestinal integrity, while considering that omega-3 polyunsaturated fatty acids play an important role in regulating these factors, consumption/supplementation of omega-3 fatty acids in endometriosis patients could be

beneficial in limiting disease progression. Some common foods that are rich in omega-3 fatty acids include fish (especially cold-water fatty fish like salmon, tuna, and herring), nuts and seeds such as flaxseed, chia seeds, and walnuts, and plant oils such as flaxseed oil and soybean oil (NIH office of dietary supplements, 2022). A diet rich in omega-3 fatty acids and fiber could help alleviate inflammation and protect a patient from lesion development and growth.

Furthermore, a diet rich in antioxidants, often found in fruits and vegetables, can help combat oxidative stress in the body. Oxidative stress, characterized by an imbalance between the production of reactive oxygen species and the body's ability to neutralize them, has been linked to the development and progression of endometriosis (Scutiero et al. 2017). Because of the correlations between reactive oxygen species and factors that drive endometriosis progression, peritoneal production of reactive oxygen species may be involved in endometriosis. Evidence has shown that oxidative stress activity and reactive oxygen species levels are high in endometriosis, and their effects translate into tissue damage, disease progression, and possible infertility (Scutiero et al. 2017; Tosti et al. 2015). Oxidative stress can be combated with antioxidant supplementation, either through foods consumed in diet or a medicinal antioxidant supplement. Studies have shown that antioxidant use can improve patient outcomes in endometriosis. For example, evidence has shown that vitamin E, vitamin C, and vitamin D can reduce endometriotic lesion growth (Sinha et al. 2017). Researchers investigated the roles of vitamins E and C in endometriosis progression through giving a vitamin E and C supplement to 46 women for two months, which resulted in 43% of the patients reporting a reduction in chronic pelvic pain and no change in the control group, as well as a reduced lesion size in the

experimental group. Vitamin D is another compound with antioxidant and anti-inflammatory properties, like vitamins E and C, and low vitamin D levels have shown to be associated with increased risk of endometriosis and increased severity of symptoms. A study has reported reduced pelvic pain after supplementation with vitamin D (Qui et al. 2020). Vitamins E, C, and D can be given as an oral supplement, or patients can get enough of this available vitamin through nutrition. Vitamin E is especially concentrated in plant-based oils, nuts, seeds, fruits, and vegetables such as almonds, spinach, pumpkin, red bell pepper, asparagus, mangoes, and avocados, while high concentrations of vitamin C are found in citrus fruits such as oranges as well as cruciferous vegetables. Unlike vitamins E and C, vitamin D is not commonly found in many natural foods. Our bodies convert the precursor, 7-dehydrocholesterol, to vitamin D when the skin absorbs UV-B radiation, though some foods including fish and some dairy products are fortified with vitamin D. More research needs to be conducted regarding the correlation between antioxidants and endometrial lesion reduction; however, results are promising, and more emphasis should be placed on the importance of dietary antioxidant consumption for endometriosis due to its positive effect.

Considering the correlations between the gut microbiome and endometriosis and how supplementation of antioxidants, anti-inflammatory compounds, and dietary fiber to promote short chain fatty acid production can inhibit endometriosis progression, and how dysbiosis of the gut microbiome can promote progression, it is important to take actions to stimulate a healthy intestinal environment. Supplementation with probiotics could be beneficial for endometriosis patients because probiotics help restore the composition of the gut microbiome and introduce beneficial functions to gut microbial communities to

prevent inflammation (Hemarajata and Versalovic, 2012). Probiotics can help stimulate a healthy environment but can also modulate patterns of gene expression to prevent inflammation in the future, according to a cohort study on humans (Van Baarlen et al. 2011). The typical Western diet consists of high levels of unhealthy fats and sugars, which has a direct effect on the gut microbiome in that this diet has been shown to cause disrupted ratio of Firmicutes/Bacteroidetes and dysbiosis in the diversity of the gut microbiome, which have shown to cause inflammation (Hemarajata and Versalovic, 2012). In general, a typical Western diet consisting of high saturated and trans fats, added sugars, red meats, and processed foods negatively alters gut microbiota. Production of biologically active metabolites such as short chain fatty acids rely on a healthy and diverse microbiome, and in turn, production of these important compounds may induce changes in microbial composition to help promote a healthy gut. In order to 'fix' the gut microbiome, probiotics should be added into the diet. Probiotics are defined as living microorganisms, which when administered in adequate amounts confer health benefits on the host (Food and Agriculture Organization of the United Nations, 2006). Probiotics help promote a healthy gut through manipulation of intestinal microbial communities, suppression of pathogens, immunomodulation, stimulation of epithelial cell proliferation, and fortification of the intestinal barrier (Hemarajata and Versalovic, 2012). Because of their beneficial mechanisms, probiotics have been proposed as a therapeutic approach to treat and prevent gynecological diseases like endometriosis. Multiple studies have demonstrated positive effects of probiotics on pain levels in endometriosis patients. A clinical trial involving 47 patients with stage 3 and 4 endometriosis compared the effects of supplementing with different *Lactobacillus* species against a control group who was

given placebo, resulting in significant pain improvement for the patients who received the probiotics (Khodaverdi et al. 2019). Similarly, another study involving 62 patients demonstrated similar outcomes in pain intensity (Khodaverdi et al. 2019). These studies support the hypothesis that probiotics reduce painful inflammation as well as modify the production of neurotransmitters implicated in pain perception (Norfuad et al. 2023). Other studies reveal that probiotics can even promote healing of existing endometriosis, as shown in an experiment with several rat models in which two rats were completely healed after supplementation while the rest improved greatly, suggesting that probiotics can enhance the healing of endometriosis lesions (Uchida and Kobayashi, 2013). Further research is necessary to establish a definite direct relationship between probiotic supplementation and endometriosis progression; however, results are promising. Endometriosis patients should consider eating foods rich in probiotics including yogurt, tempeh, kimchi, kombucha, and vinegars to build a healthy microbial community.

As discussed, there are several nutritional supplements that can help alleviate endometriosis symptoms and protect against progression, but there are also several foods and nutritional components to avoid including foods high in saturated and trans-fat, processed food, excess added sugars, and red meat, which are all examples of pro-inflammatory foods. Because the typical Western diet is high in all these components, endometriosis patients should be especially mindful of what they eat and should consider alternative, primarily plant-based diets. A study conducted in 2010 found that regular intake of palmitic acid, which is a saturated fatty acid derived from meat and dairy products, and trans-fat was associated with increased risk of endometriosis, while comparatively total fat consumption did not confer the same risk (Missmer et al. 2010).

Additionally, because endometriosis is an estrogen dependent disorder, it is important to consider dietary factors that modulate estrogen levels. For example, studies have shown that reducing dietary fat, reducing meat consumption, and increasing dietary fiber results in reduced circulating estrogen levels and maintenance of hormonal balance (Barnard et al. 2023).

Due to the unhealthy fat content, makeup of pro-inflammatory components, and processed nature of meat, specifically red meat, a plant-based diet may be beneficial for endometriosis patients. Several studies have suggested that red meat consumption increases the risk of developing endometriosis due to its association with inflammation, gut microbiome dysbiosis, and higher levels of estradiol (Barnard et al. 2023). For example, a study tested the hypothesis that red meat consumption influences endometriosis risk and found that women consuming more than two servings of red meat per day had a 56% greater risk of endometriosis compared with those consuming less than one serving of red meat per week (Yamamoto et al. 2018). This study evaluated the results of red meat consumption alongside consumption of poultry and fish, though risk of poultry consumption was not nearly as significant compared to red meat and consumption of fish showed minimal risk. To promote a healthy microbial environment in the gut to reduce risk for and further progression of endometriosis, diet should be rich in whole foods and anti-inflammatory components. When considering a diet that reduces dietary fat consumption while increasing dietary fiber to regulate short chain fatty acid production and estrogen levels, a plant-based diet is likely the best choice. A study was conducted comparing a plant-based diet to an omnivorous diet and results showed that individuals on a plant-based diet had more anti-inflammatory compounds in the gut

microbiome than the latter (Craig et al. 2021). A similar study conducted a cross-over trial between a low-fat vegan diet and a placebo for endometriosis patients, resulting in lower pain levels for the individuals on the plant-based diet as well as reduced estrogen activity, providing evidence that a plant-based diet could be used as treatment for endometriosis (Barnard, 2000). The benefits of a plant-based diet for endometriosis can be understood through the anti-inflammatory properties and modulation of hormonal balance, but also through the promotion of a healthy gut microbiome which as discussed throughout this review is extremely beneficial. A plant based diet rich in fruits, vegetables, and soy and low in saturated/trans-fat is beneficial to the gut microbiome through promotion of a more diverse microbial system, regulated distribution of species including a healthy Firmicutes/ Bacteroidetes ratio, increased nutrient bioavailability to the lower gastrointestinal tract promoting a more healthy microbiome, high intake of fiber which promotes short chain fatty acid production, increased intake of phytoestrogens through soy which regulate circulating estrogen levels, and high intake of essential vitamins with anti-inflammatory properties (Tomova et al. 2019). Overall, there are numerous reasons why a woman should adopt a plant-based diet or incorporate more plants and less meat to reduce the risk of endometriosis, to treat symptoms of endometriosis, and to minimize or even reverse lesion growth.

Because many nutritional factors have the potential to impact progression of endometriosis, it is important for endometriosis patients to be mindful of what their diets consist of. Consuming foods high in fiber to promote short chain fatty acid production, foods rich in omega-3 fatty acids such as fish and walnuts, as well as consuming foods

rich in antioxidants including diverse fruits and vegetables is a feasible, personalized approach to manage pain and to protect against disease progression.

Conclusion

Endometriosis is a common gynecological condition driven by estrogen and inflammation and may cluster in families due to polygenic inheritance. Dysbiosis of the gut has shown to be associated with endometriosis considering the microbiome's influence on inflammatory response, immune response, and estrogen cycling, along with associations between short chain fatty acid production, intestinal permeability, and endometriosis risk. Fecal metabolomic studies to characterize the gut microbiome and identify patterns of microbes associated with endometriosis may be an effective and non-invasive avenue to diagnose endometriosis. Considering the relationship between endometriosis and the gut microbiome, a nutrition-based treatment approach including lots of fiber to promote short chain fatty acid production, foods containing phytoestrogens, polyunsaturated fatty acids, antioxidants, and probiotic supplements may be effective.

Further research in women's medicine and exploring the connections between the gut microbiome and health conditions such as endometriosis is vital for advancing our knowledge of women's health and improving diagnostic, preventative, and treatment approaches. Such research can lead to innovative therapeutic strategies, a better understanding of gender-specific health concerns, and personalized interventions to enhance women's overall well-being.

References

1. Alimi Y, Iwanaga J, Loukas M, Tubbs RS. The Clinical Anatomy of Endometriosis: A Review. Cureus. 2018 Sep 25;10(9):e3361. doi: 10.7759/cureus.3361. PMID: 30510871; PMCID: PMC6257623.

2. Amaya SC, Savaris RF, Filipovic CJ, Wise JD, Hestermann E, Young SL, Lessey BA. Resveratrol and endometrium: a closer look at an active ingredient of red wine using in vivo and in vitro models. Reprod Sci. 2014 Nov;21(11):1362-9. doi: 10.1177/1933719114525271. Epub 2014 Mar 6. PMID: 24604232; PMCID: PMC4212329.

3. Annika S, Sajal G. The Role of Antioxidant Supplementation in Endometriosis Therapy. J Gynecol Women's Health. 2017; 3(1): 555601. DOI: 10.19080/JGWH.2017.03.555601.

4. Arnone AA, Cook KL. Gut and Breast Microbiota as Endocrine Regulators of Hormone Receptor-positive Breast Cancer Risk and Therapy Response. Endocrinology. 2022 Nov 14;164(1):bqac177. doi: 10.1210/endocr/bqac177. PMID: 36282876; PMCID: PMC9923803.

5. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims

S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J; MetaHIT Consortium; Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F, Huber W, van Hylckama-Vlieg J, Jamet A, Juste C, Kaci G, Knol J, Lakhdari O, Layec S, Le Roux K, Maguin E, Mérieux A, Melo Minardi R, M'rini C, Muller J, Oozeer R, Parkhill J, Renault P, Rescigno M, Sanchez N, Sunagawa S, Torrejon A, Turner K, Vandemeulebrouck G, Varela E, Winogradsky Y, Zeller G, Weissenbach J, Ehrlich SD, Bork P. Enterotypes of the human gut microbiome. Nature. 2011 May 12;473(7346):174-80. doi: 10.1038/nature09944. Epub 2011 Apr 20. Erratum in: Nature. 2011 Jun 30;474(7353):666. Erratum in: Nature. 2014 Feb 27;506(7489):516. PMID: 21508958; PMCID: PMC3728647.

6. Ashwitha Acharya, Shilpa S. Shetty, Suchetha Kumari N, Role of gut microbiota derived short chain fatty acid metabolites in modulating female reproductive health, Human Nutrition & Metabolism, Volume 36, 2024, 200256, ISSN 2666-1497, https://doi.org/10.1016/j.hnm.2024.200256

.

7. Ata, B., Yildiz, S., Turkgeldi, E. et al. The Endobiota Study: Comparison of Vaginal, Cervical and Gut Microbiota Between Women with Stage 3/4 Endometriosis and Healthy Controls. Sci Rep 9, 2204 (2019). https://doi.org/10.1038/s41598-019-39700-6

8. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen-gut microbiome axis: Physiological and clinical implications. Maturitas. 2017 Sep;103:45-53. doi: 10.1016/j.maturitas.2017.06.025. Epub 2017 Jun 23. PMID: 28778332.

9. Barnard ND, Holtz DN, Schmidt N, Kolipaka S, Hata E, Sutton M, Znayenko-Miller T, Hazen ND, Cobb C, Kahleova H. Nutrition in the prevention and treatment of endometriosis: A review. Front Nutr. 2023 Feb 17;10:1089891. doi: 10.3389/fnut.2023.1089891. PMID: 36875844; PMCID: PMC9983692.

10. Barnard ND, Scialli AR, Hurlock D, Bertron P. Diet and sex-hormone binding globulin, dysmenorrhea, and premenstrual symptoms. Obstet Gynecol. 2000 Feb;95(2):245-50. doi: 10.1016/s0029-7844(99)00525-6. PMID: 10674588.

11. Bauermeister A, Mannochio-Russo H, Costa-Lotufo LV, Jarmusch AK, Dorrestein PC. Mass spectrometry-based metabolomics in microbiome investigations. Nat Rev Microbiol. 2022 Mar;20(3):143-160. doi: 10.1038/s41579-021-00621-9. Epub 2021 Sep 22. PMID: 34552265; PMCID: PMC9578303.

12. Bhatt B, Zeng P, Zhu H, Sivaprakasam S, Li S, Xiao H, Dong L, Shiao P, Kolhe R, Patel N, et al (2018) Gpr109a limits microbiota-induced IL-23 production to constrain ILC3-mediated colonic inflammation. J Immunol 200: 2905–2914. doi:10.4049/jimmunol.1701625

13. Bulletti C, Coccia ME, Battistoni S, Borini A. Endometriosis and infertility. J Assist Reprod Genet. 2010 Aug;27(8):441-7. doi: 10.1007/s10815-010-9436-1. Epub 2010 Jun 25. PMID: 20574791; PMCID: PMC2941592.

14. Camilleri M. Leaky gut: mechanisms, measurement and clinical implications in humans. Gut. 2019 Aug;68(8):1516-1526. doi: 10.1136/gutjnl-2019-318427. Epub 2019 May 10. PMID: 31076401; PMCID: PMC6790068.

15. Chadchan, S.B., Naik, S.K., Popli, P. et al. Gut microbiota and microbiota-derived metabolites promote endometriosis. Cell Death Discov. 9, 28 (2023). https://doi.org/10.1038/s41420-023-01309-0

16. Chadchan SB, Popli P, Ambati CR, Tycksen E, Han SJ, Bulun SE, Putluri N, Biest SW, Kommagani R. Gut microbiota-derived short-chain fatty acids protect against the progression of endometriosis. Life Sci Alliance. 2021 Sep 30;4(12):e202101224. doi: 10.26508/lsa.202101224. PMID: 34593556; PMCID: PMC8500332.

17. Chantalat E, Valera MC, Vaysse C, Noirrit E, Rusidze M, Weyl A, Vergriete K, Buscail E, Lluel P, Fontaine C, Arnal JF, Lenfant F. Estrogen Receptors and Endometriosis. Int J Mol Sci. 2020 Apr 17;21(8):2815. doi: 10.3390/ijms21082815. PMID: 32316608; PMCID: PMC7215544.

18. Corbett GA, Lee S, Woodruff TJ. Nutritional interventions to ameliorate the effect of endocrine disruptors on human reproductive health: A semi-structured review from FIGO. Int J Gynecol Obstet. 2022; 157: 489–501. doi: 10.1002/ijgo.14126

19. Covens AL, Christopher P, Casper RF. The effect of dietary supplementation with fish oil fatty acids on surgically induced endometriosis in the rabbit. Fertil Steril. 1988 Apr;49(4):698-703. doi: 10.1016/s0015-0282(16)59842-2. PMID: 2832216.

20. Craig WJ, Mangels AR, Fresán U, Marsh K, Miles FL, Saunders AV, Haddad EH, Heskey CE, Johnston P, Larson-Meyer E, Orlich M. The Safe and Effective Use of Plant-Based Diets with Guidelines for Health Professionals. Nutrients. 2021 Nov 19;13(11):4144. doi: 10.3390/nu13114144. PMID: 34836399; PMCID: PMC8623061.

21. Dastur AE, Tank PD. John A Sampson and the origins of Endometriosis. J Obstet Gynaecol India. 2010 Aug;60(4):299–300. doi: 10.1007/s13224-010-0046-8. Epub 2011 Mar 13. PMCID: PMC3394535.

22. Domínguez-López I, Yago-Aragón M, Salas-Huetos A, Tresserra-Rimbau A, Hurtado-Barroso S. Effects of Dietary Phytoestrogens on Hormones throughout a Human Lifespan: A Review. Nutrients. 2020 Aug 15;12(8):2456. doi: 10.3390/nu12082456. PMID: 32824177; PMCID: PMC7468963.

23. Dun, E.C., Taylor, R.N. & Wieser, F. Advances in the genetics of endometriosis. Genome Med 2, 75 (2010). https://doi.org/10.1186/gm196

24. Dutta S, Banu SK, Arosh JA. Endocrine disruptors and endometriosis. Reprod Toxicol. 2023 Jan;115:56-73. doi: 10.1016/j.reprotox.2022.11.007. Epub 2022 Nov 24. PMID: 36436816.

25. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, Flavell RA. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell. 2011 May 27;145(5):745-57. doi: 10.1016/j.cell.2011.04.022. Epub 2011 May 12. PMID: 21565393; PMCID: PMC3140910.

26. Fu Y, Wang Y, Gao H, Li D, Jiang R, Ge L, Tong C, Xu K. Associations among Dietary Omega-3 Polyunsaturated Fatty Acids, the Gut Microbiota, and Intestinal Immunity. Mediators Inflamm. 2021 Jan 2;2021:8879227. doi: 10.1155/2021/8879227. PMID: 33488295; PMCID: PMC7801035.

27. Gao J, Xu Y, Zhong T, Yu X, Wang L, Xiao Y, Peng Y, Sun Q. A review of food contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin and its toxicity associated with metabolic disorders. Curr Res Food Sci. 2023 Oct 10;7:100617. doi: 10.1016/j.crfs.2023.100617. PMID: 37881334; PMCID: PMC10594546.

28. Gao S, Sun R, Singh R, Yu So S, Chan CTY, Savidge T, Hu M. The role of gut microbial β-glucuronidase in drug disposition and development. Drug Discov Today. 2022 Oct;27(10):103316. doi: 10.1016/j.drudis.2022.07.001. Epub 2022 Jul 9. PMID: 35820618; PMCID: PMC9717552.

29. Hadfield R, Mardon H, Barlow D, Kennedy S. Delay in the diagnosis of endometriosis: a survey of women from the USA and the UK. Hum Reprod. 1996 Apr;11(4):878-80. doi: 10.1093/oxfordjournals.humrep.a019270. PMID: 8671344.

30. Hansen KA, Eyster KM. Genetics and genomics of endometriosis. Clin Obstet Gynecol. 2010 Jun;53(2):403-12. doi: 10.1097/GRF.0b013e3181db7ca1. PMID: 20436317; PMCID: PMC4346178.

31. Heerdt AS, Young CW, Borgen PO, Calcium glucarate as a chemopreventive agent in breast cancer. Israel Journal of Medical Sciences. 31(2-3): 101-5, 1995.

32. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therap Adv Gastroenterol. 2013 Jan;6(1):39-51. doi: 10.1177/1756283X12459294. PMID: 23320049; PMCID: PMC3539293.

33. Hrncir T. Gut Microbiota Dysbiosis: Triggers, Consequences, Diagnostic and Therapeutic Options. Microorganisms. 2022 Mar 7;10(3):578. doi: 10.3390/microorganisms10030578. PMID: 35336153; PMCID: PMC8954387.

34. Hsu AL, Khachikyan I, Stratton P. Invasive and noninvasive methods for the diagnosis of endometriosis. Clin Obstet Gynecol. 2010 Jun;53(2):413-9. doi: 10.1097/GRF.0b013e3181db7ce8. PMID: 20436318; PMCID: PMC2880548.

35. Interdonato L, Siracusa R, Fusco R, Cuzzocrea S, Di Paola R. Endocrine Disruptor Compounds in Environment: Focus on Women's Reproductive Health and Endometriosis. Int J Mol Sci. 2023 Mar 16;24(6):5682. doi: 10.3390/ijms24065682. PMID: 36982755; PMCID: PMC10058284.

36. Kanellopoulos D, Karagianni D, Pergialiotis V, Nikiteas N, Lazaris AC, Iliopoulos D. Endometriosis and Subfertility: A Literature Review. Maedica (Bucur). 2022 Jun;17(2):458-463. doi: 10.26574/maedica.2022.17.2.458. PMID: 36032627; PMCID: PMC9375884.

37. Karu N, Deng L, Slae M, Guo AC, Sajed T, Huynh H, Wine E, Wishart DS. A review on human fecal metabolomics: Methods, applications and the human fecal metabolome database. Anal Chim Acta. 2018 Nov 7;1030:1-24. doi: 10.1016/j.aca.2018.05.031. Epub 2018 May 12. PMID: 30032758.

38. Kennedy S, Hadfield, Barlow D, Weeks DE, Laird E, Golding S. Use of MRI in genetic studies of endometriosis. Am J Med Genet. 1997;71:371–372.

39. Khodaverdi S, Mohammadbeigi R, Khaledi M, Mesdaghinia L, Sharifzadeh F, Nasiripour S, Gorginzadeh M. Beneficial Effects of Oral Lactobacillus on Pain Severity in Women Suffering from Endometriosis: A Pilot Placebo-Controlled Randomized Clinical Trial. Int J Fertil Steril. 2019 Oct;13(3):178-183. doi: 10.22074/ijfs.2019.5584. Epub 2019 Jul 14. PMID: 31310070; PMCID: PMC6642422.

40. Khoo SH, Al-Rubeai M. Metabolomics as a complementary tool in cell culture. Biotechnol Appl Biochem. 2007 Jun;47(Pt 2):71-84. doi: 10.1042/BA20060221. PMID: 17492944.

41. Kosiewicz MM, Zirnheld AL, Alard P. Gut microbiota, immunity, and disease: a complex relationship. Front Microbiol. 2011 Sep 5;2:180. doi: 10.3389/fmicb.2011.00180. PMID: 21922015; PMCID: PMC3166766.

42. Kunst C, Schmid S, Michalski M, Tümen D, Buttenschön J, Müller M, Gülow K. The Influence of Gut Microbiota on Oxidative Stress and the Immune System. Biomedicines. 2023 May 8;11(5):1388. doi: 10.3390/biomedicines11051388. PMID: 37239059; PMCID: PMC10216031.

43. Liebisch G, Ecker J, Roth S, Schweizer S, O¨ttl V, Scho¨tt HF, Yoon H, Haller D, Holler E, Burkhardt R, et al (2019) Quantification of fecal short chain fatty acids by liquid chromatography tandem mass spectrometry- investigation of pre-analytic stability. Biomolecules 9: 121. doi:10.3390/ biom9040121

44. López-Moreno, A.; Torres-Sánchez, A.; Acuña, I.; Suárez, A.; Aguilera, M. Representative Bacillus sp. AM1 from Gut Microbiota Harbor Versatile Molecular Pathways for Bisphenol A Biodegradation. Int. J. Mol. Sci. 2021, 22, 4952. https://doi.org/10.3390/ijms22094952

45. Maddern J, Grundy L, Castro J, Brierley SM. Pain in Endometriosis. Front Cell Neurosci. 2020 Oct 6;14:590823. doi: 10.3389/fncel.2020.590823. PMID: 33132854; PMCID: PMC7573391.

46. Mahsa Tanha, Mahmood Bozorgmehr, Mohammad-Reza Shokri, Haleh Edalatkhah, Mahya Tanha, Amir-Hassan Zarnani, Shohreh Nikoo, 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin potential impacts on peripheral blood mononuclear cells of endometriosis women, Journal of Reproductive Immunology, Volume 149, 2022, 103439, ISSN 0165-0378, https://doi.org/10.1016/j.jri.2021.103439.

47. Messer, Jeannette and Chang, Eugene. Physiology of the Gastrointestinal Tract, sixth edition, 36.5.2: Dysbiosis, 2018, Vol. 1. https://www.sciencedirect.com/topics/medicine-and-

dentistry/dysbiosis#:~:text=Dysbiosis%20is%20the%20substitution%20of,systemic%20chronic%20inflammation%20%5B14%5D.

48. Missmer SA, Chavarro JE, Malspeis S, Bertone-Johnson ER, Hornstein MD, Spiegelman D, Barbieri RL, Willett WC, Hankinson SE. A prospective study of dietary fat consumption and endometriosis risk. Hum Reprod. 2010 Jun;25(6):1528-35. doi: 10.1093/humrep/deq044. Epub 2010 Mar 23. PMID: 20332166; PMCID: PMC2873173.

49. Moradi Y, Shams-Beyranvand M, Khateri S, Gharahjeh S, Tehrani S, Varse F, Tiyuri A, Najmi Z. A systematic review on the prevalence of endometriosis in women. Indian J Med Res. 2021 Mar;154(3):446-454. doi: 10.4103/ijmr.IJMR_817_18. PMID: 35345070; PMCID: PMC9131783.

50. Mroczyńska M, Libudzisz Z. Beta-glucuronidase and beta-glucosidase activity of Lactobacillus and Enterococcus isolated from human feces. Pol J Microbiol. 2010;59(4):265-9. PMID: 21466044.

51. Nabi MY, Nauhria S, Reel M, Londono S, Vasireddi A, Elmiry M, Ramdass PVAK. Endometriosis and irritable bowel syndrome: A systematic review and meta-analyses. Front Med (Lausanne). 2022 Jul 25;9:914356. doi: 10.3389/fmed.2022.914356. PMID: 35957857; PMCID: PMC9357916.

52. Ni Z, Sun S, Bi Y, Ding J, Cheng W, Yu J, Zhou L, Li M, Yu C. Correlation of fecal metabolomics and gut microbiota in mice with endometriosis. Am J Reprod Immunol. 2020 Dec;84(6):e13307. doi: 10.1111/aji.13307. Epub 2020 Aug 4. PMID: 32681566.

53. NIH Office of Dietary Supplements, Omega-3 Fatty Acids, fact sheet for consumers, 2022, https://ods.od.nih.gov/factsheets/Omega3FattyAcids-Consumer/

54. Norfuad FA, Mokhtar MH, Nur Azurah AG. Beneficial Effects of Probiotics on Benign Gynaecological Disorders: A Review. Nutrients. 2023 Jun 13;15(12):2733. doi: 10.3390/nu15122733. PMID: 37375637; PMCID: PMC10301998.

55. Ortiz CN, Torres-Reverón A, Appleyard CB. Metabolomics in endometriosis: challenges and perspectives for future studies. Reprod Fertil. 2021 May 6;2(2):R35-R50. doi: 10.1530/RAF-20-0047. PMID: 35128453; PMCID: PMC8812441.

56. Paradis T, Bègue H, Basmaciyan L, Dalle F, Bon F. Tight Junctions as a Key for Pathogens Invasion in Intestinal Epithelial Cells. Int J Mol Sci. 2021 Mar 2;22(5):2506. doi: 10.3390/ijms22052506. PMID: 33801524; PMCID: PMC7958858.

57. Parazzini F, Chiaffarino F, Surace M, Chatenoud L, Cipriani S, Chiantera V, Benzi G, Fedele L. Selected food intake and risk of endometriosis. Hum Reprod. 2004 Aug;19(8):1755-9. doi: 10.1093/humrep/deh395. Epub 2004 Jul 14. PMID: 15254009.

58. Peivasteh-Roudsari L, Barzegar-Bafrouei R, Sharifi KA, Azimisalim S, Karami M, Abedinzadeh S, Asadinezhad S, Tajdar-Oranj B, Mahdavi V, Alizadeh AM, Sadighara P, Ferrante M, Conti GO, Aliyeva A, Mousavi Khaneghah A. Origin, dietary exposure, and toxicity of endocrine-disrupting food chemical contaminants: A comprehensive review. Heliyon. 2023 Jul 11;9(7):e18140. doi: 10.1016/j.heliyon.2023.e18140. PMID: 37539203; PMCID: PMC10395372.

59. Qi X, Yun C, Pang Y, Qiao J. The impact of the gut microbiota on the reproductive and metabolic endocrine system. Gut Microbes. 2021 Jan-Dec;13(1):1-21. doi: 10.1080/19490976.2021.1894070. PMID: 33722164; PMCID: PMC7971312.

60. Qiu Y, Yuan S, Wang H. Vitamin D status in endometriosis: a systematic review and meta-analysis. Arch Gynecol Obstet. 2020 Jul;302(1):141-152. doi: 10.1007/s00404-020-05576-5. Epub 2020 May 19. PMID: 32430755.

61. Rahman, Azizur, Parnian Baharlouei, Eleanor Hui Yan Koh, Diana Gabby Pirvu, Rameesha Rehmani, Mateo Arcos, and Simron Puri. 2024. "A Comprehensive Analysis of Organic Food: Evaluating Nutritional Value and Impact on Human Health" Foods 13, no. 2: 208. https://doi.org/10.3390/foods13020208

62. Ranney B. Endometriosis. IV. Hereditary Tendency. Obstet Gynecol. 1971;37(5):734–737.

63. Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fundam Appl Toxicol. 1993 Nov;21(4):433-41. doi: 10.1006/faat.1993.1119. PMID: 8253297.

64. Romain Daillère, Marie Vétizou, Nadine Waldschmitt, Takahiro Yamazaki, Christophe Isnard, Vichnou Poirier-Colame, Connie P.M. Duong, Caroline Flament, Patricia Lepage, Maria Paula Roberti, Bertrand Routy, Nicolas Jacquelot, Lionel Apetoh, Sonia Becharef, Sylvie Rusakiewicz, Philippe Langella, Harry Sokol, Guido Kroemer, David Enot, Antoine Roux, Alexander Eggermont, Eric Tartour, Ludger Johannes, Paul-Louis Woerther, Elisabeth Chachaty, Jean-Charles Soria, Encouse Golden, Silvia Formenti, Magdalena Plebanski, Mutsa Madondo, Philip Rosenstiel, Didier Raoult, Vincent Cattoir, Ivo Gomperts Boneca, Mathias Chamaillard, Laurence Zitvogel, Enterococcus hirae and Barnesiella intestinihominis Facilitate Cyclophosphamide-Induced Therapeutic Immunomodulatory Effects, Immunity, Volume 45, Issue 4, 2016, Pages 931-943, ISSN 1074-7613, https://doi.org/10.1016/j.immuni.2016.09.009

65. Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components. Eur J Nutr. 2018 Feb;57(1):1-24. doi: 10.1007/s00394-017-1445-8. Epub 2017 Apr 9. PMID: 28393285; PMCID: PMC5847071.

66. Scutiero G, Iannone P, Bernardi G, Bonaccorsi G, Spadaro S, Volta CA, Greco P, Nappi L. Oxidative Stress and Endometriosis: A Systematic Review of the Literature. Oxid Med Cell Longev. 2017;2017:7265238. doi: 10.1155/2017/7265238. Epub 2017 Sep 19. PMID: 29057034; PMCID: PMC5625949.

67. Shaikh SD, Sun N, Canakis A, Park WY, Weber HC. Irritable Bowel Syndrome and the Gut Microbiome: A Comprehensive Review. J Clin Med. 2023 Mar 28;12(7):2558. doi: 10.3390/jcm12072558. PMID: 37048642; PMCID: PMC10095554.

68. Simpson J, Elias S, Malinak L, et al. Heritable aspects of endometriosis: 1. Genetic studies. Am J Obstet Gynecol. 1980;137:327–331.

69. Skar V, Skar AG, Strømme JH. Beta-glucuronidase activity related to bacterial growth in common bile duct bile in gallstone patients. Scand J Gastroenterol. 1988 Jan;23(1):83-90. doi: 10.3109/00365528809093853. PMID: 3344403.

70. Smolarz B, Szyłło K, Romanowicz H. Endometriosis: Epidemiology, Classification, Pathogenesis, Treatment and Genetics (Review of Literature). Int J Mol Sci. 2021 Sep 29;22(19):10554. doi: 10.3390/ijms221910554. PMID: 34638893; PMCID: PMC8508982.

71. Stephens Victoria R. , Rumph Jelonia T. , Ameli Sharareh , Bruner-Tran Kaylon L. , Osteen Kevin G. The Potential Relationship Between Environmental Endocrine Disruptor

Exposure and the Development of Endometriosis and Adenomyosis. Frontiers in Physiology, vol.12, 2022. doi:10.3389/fphys.2021.807685. Issn: 1664-042X. https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2021.807685

72. Sudipta Dutta, Sakhila K. Banu, Joe A. Arosh, Endocrine disruptors and endometriosis, Reproductive Toxicology, Volume 115, 2023, Pages 56-73, ISSN 0890-6238, https://doi.org/10.1016/j.reprotox.2022.11.007.

73. Talwar C, Singh V, Kommagani R. The gut microbiota: a double-edged sword in endometriosis†. Biol Reprod. 2022 Oct 11;107(4):881-901. doi: 10.1093/biolre/ioac147. PMID: 35878972; PMCID: PMC9562115.

74. Tomio K, Kawana K, Taguchi A, Isobe Y, Iwamoto R, Yamashita A, Kojima S, Mori M, Nagamatsu T, Arimoto T, Oda K, Osuga Y, Taketani Y, Kang JX, Arai H, Arita M, Kozuma S, Fujii T. Omega-3 polyunsaturated Fatty acids suppress the cystic lesion formation of peritoneal endometriosis in transgenic mouse models. PLoS One. 2013 Sep 10;8(9):e73085. doi: 10.1371/journal.pone.0073085. PMID: 24039864; PMCID: PMC3769312.

75. Tomova A, Bukovsky I, Rembert E, Yonas W, Alwarith J, Barnard ND, Kahleova H. The Effects of Vegetarian and Vegan Diets on Gut Microbiota. Front Nutr. 2019 Apr 17;6:47. doi: 10.3389/fnut.2019.00047. PMID: 31058160; PMCID: PMC6478664.

76. Tosti C, Pinzauti S, Santulli P, Chapron C, Petraglia F. Pathogenetic Mechanisms of Deep Infiltrating Endometriosis. Reprod Sci. 2015 Sep;22(9):1053-9. doi: 10.1177/1933719115592713. Epub 2015 Jul 12. PMID: 26169038.

77. Treloar SA, O'Connor DT, O'Connor VM, Martin NG. Genetic influences on endometriosis in an Australian twin sample. sueT@qimr.edu.au. Fertil Steril. 1999 Apr;71(4):701-10. doi: 10.1016/s0015-0282(98)00540-8. PMID: 10202882.

78. Troisi J, Mollo A, Lombardi M, Scala G, Richards SM, Symes SJK, Travaglino A, Neola D, de Laurentiis U, Insabato L, Di Spiezio Sardo A, Raffone A, Guida M. The Metabolomic Approach for the Screening of Endometrial Cancer: Validation from a Large Cohort of Women Scheduled for Gynecological Surgery. Biomolecules. 2022 Sep 2;12(9):1229. doi: 10.3390/biom12091229. PMID: 36139068; PMCID: PMC9496630.

79. Uchida M, Kobayashi O. Effects of Lactobacillus gasseri OLL2809 on the induced endometriosis in rats. Biosci Biotechnol Biochem. 2013;77(9):1879-81. doi: 10.1271/bbb.130319. Epub 2013 Sep 7. PMID: 24018664.

80. Ugwumadu L, Chakrabarti R, Williams-Brown E, Rendle J, Swift I, John B, Allen-Coward H, Ofuasia E. The role of the multidisciplinary team in the management of deep infiltrating endometriosis. Gynecol Surg. 2017;14(1):15. doi: 10.1186/s10397-017-1018-0. Epub 2017 Aug 15. PMID: 28890677; PMCID: PMC5570783.

81. Van Baarlen P, Troost F, van der Meer C, Hooiveld G, Boekschoten M, Brummer RJ, Kleerebezem M. Human mucosal in vivo transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. Proc Natl Acad Sci U S A. 2011 Mar 15;108 Suppl 1(Suppl 1):4562-9. doi: 10.1073/pnas.1000079107. Epub 2010 Sep 7. PMID: 20823239; PMCID: PMC3063594.

82. Vannuccini S, Clemenza S, Rossi M, Petraglia F. Hormonal treatments for endometriosis: The endocrine background. Rev Endocr Metab Disord. 2022 Jun;23(3):333-355. doi: 10.1007/s11154-021-09666-w. Epub 2021 Aug 17. PMID: 34405378; PMCID: PMC9156507.

83. Van Stein K, Schubert K, Ditzen B, Weise C. Understanding Psychological Symptoms of Endometriosis from a Research Domain Criteria Perspective. J Clin Med. 2023 Jun 15;12(12):4056. doi: 10.3390/jcm12124056. PMID: 37373749; PMCID: PMC10299570.

84. Walaszek Z. Potential use of D-glucaric acid derivatives in cancer prevention. Cancer Lett. 1990 Oct 8;54(1-2):1-8. doi: 10.1016/0304-3835(90)90083-a. PMID: 2208084.

85. Warner GR, Flaws JA. Bisphenol A and Phthalates: How Environmental Chemicals Are Reshaping Toxicology. Toxicol Sci. 2018 Dec 1;166(2):246-249. doi: 10.1093/toxsci/kfy232. PMID: 30496570; PMCID: PMC6260148.

86. Wishart DS. Metabolomics for Investigating Physiological and Pathophysiological Processes. Physiol Rev. 2019 Oct 1;99(4):1819-1875. doi: 10.1152/physrev.00035.2018. PMID: 31434538.

87. World Health Organization- Food and Agriculture Organization of the United Nations, 2006. https://www.fao.org/3/a0512e/a0512e.pdf

88. Xholli A, Cremonini F, Perugi I, Londero AP, Cagnacci A. Gut Microbiota and Endometriosis: Exploring the Relationship and Therapeutic Implications. Pharmaceuticals. 2023; 16(12):1696. https://doi.org/10.3390/ph16121696

89. Xiong RG, Zhou DD, Wu SX, Huang SY, Saimaiti A, Yang ZJ, Shang A, Zhao CN, Gan RY, Li HB. Health Benefits and Side Effects of Short-Chain Fatty Acids. Foods. 2022 Sep 15;11(18):2863. doi: 10.3390/foods11182863. PMID: 36140990; PMCID: PMC9498509.

90. Xu X, Duncan AM, Merz BE, Kurzer MS. Effects of soy isoflavones on estrogen and phytoestrogen metabolism in premenopausal women. Cancer Epidemiol Biomarkers Prev. 1998 Dec;7(12):1101-8. PMID: 9865428.

91. Yamamoto A, Harris HR, Vitonis AF, Chavarro JE, Missmer SA. A prospective cohort study of meat and fish consumption and endometriosis risk. Am J Obstet Gynecol. 2018

Aug;219(2):178.e1-178.e10. doi: 10.1016/j.ajog.2018.05.034. Epub 2018 Jun 2. PMID: 29870739; PMCID: PMC6066416.

92. Yang, Q.; Zhang, A.H.; Miao, J.H.; Sun, H.; Han, Y.; Yan, G.L.; Wu, F.F.; Wang, X.J. Metabolomics biotechnology, applications, and future trends: A systematic review. RSC Adv. 2019, 9, 37245–37257

93. Yang, Xing, Xie, Lu, Li, Yixue, Wei, Chaochun, More than 9,000,000 Unique Genes in Human Gut Bacterial Community: Estimating Gene Numbers Inside a Human Body, 2009, vol. 4, 10.1371/journal.pone.0006074

94. Yoo JY, Groer M, Dutra SVO, Sarkar A, McSkimming DI. Gut Microbiota and Immune System Interactions. Microorganisms. 2020 Oct 15;8(10):1587. doi: 10.3390/microorganisms8101587. Erratum in: Microorganisms. 2020 Dec 21;8(12): PMID: 33076307; PMCID: PMC7602490.

95. Yuan M, Li D, Zhang Z, Sun H, An M, Wang G. Endometriosis induces gut microbiota alterations in mice. Hum Reprod. 2018 Apr 1;33(4):607-616. doi: 10.1093/humrep/dex372. PMID: 29462324.

96. Zhao Y., Ma G., Yang X. HDAC5 promotes Mycoplasma pneumoniae-induced inflammation in macrophages through NF-κB activation. Life Sci. 2019;221:13–19. doi: 10.1016/j.lfs.2019.02.004.