

**DETERMINING THE EFFECT OF CARBON SOURCES ON *GARDNERELLA*
VAGINALIS BIOFILM FORMATION AND DESIGNING A BIOFILM DISRUPTION
INDUCER**

**INVESTIGATING THE NEED FOR BETTER INFERTILITY TREATMENT OPTIONS
FOR WOMEN**

A Thesis Prospectus
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By
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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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General Research Problem: Better Treatment for Women's Reproductive Health

Is there a need for better treatment options for different diagnoses within women's reproductive health?

Many women will run into issues related to their reproductive health at some point in their lives, ranging from menstrual issues to cancer. In most cases, there are treatment options, but are those options enough?

One of these issues is bacterial vaginosis (BV). BV is the most common vaginal infection in reproductive-age women (Machado & Cerca, 2015). African-American and Mexican American women are affected more than white women, with infection rates for African-American women being more than double that of white women (*STD Facts - Bacterial Vaginosis*, 2022). BV is an infection caused by the overgrowth of multiple pathogenic anaerobes in the vagina. *Gardnerella* is the dominant genus associated with BV, and it can form a biofilm. Compared to the planktonic or normal state, *Gardnerella* in the biofilm state is associated with decreased antibiotic susceptibility, so recurrence rates of BV remain high. Therefore, a new treatment option is needed.

In addition to causing irritating symptoms that can affect women's daily lives, BV can also cause pregnancy problems such as premature birth or a low birth weight. Because BV affects women's reproductive system, it can also decrease fertility (*Bacterial Vaginosis and Fertility | American Pregnancy Association*, n.d.).

In the United States, about 19% of women with no prior births from the age of 15 to 49 are unable to get pregnant after one year (*Infertility | Reproductive Health | CDC*, 2022). Although expensive, there are a number of infertility treatments that have developed over the years, and these treatments have a success rate of about 50% (Illiades, 2016); however, not all couples will

choose to get treatment. Treatment options include medications, surgical procedures, assisted conception, or a combination of the treatment options (*Treatment for Infertility - NHS*, 2017). Are these treatment options enough?

***Gardnerella Vaginalis* Biofilm Formation and Designing a Disruption Inducer**

How can we determine how different carbon sources affect Gardnerella Vaginalis biofilm formation and design a biofilm disruption inducer?

The goal of the technical research project is to determine the effect of the dominant carbon sources on *Gardnerella vaginalis* biofilm formation and to design a biofilm disruption inducer. Treating symptomatic BV is expensive, costing about \$4.8 billion globally each year. Symptoms include pain or burning in the vagina, burning when peeing, a thin gray vaginal discharge, and itching around the outside of the vagina (*STD Facts - Bacterial Vaginosis*, 2022). The specific cause of BV is unknown, but certain factors are known to increase the risk: douching, not using condoms, and having multiple sex partners (*STD Facts - Bacterial Vaginosis*, 2022). These factors can disrupt the normal balance of healthy vaginal bacteria.

Current treatment options include broad spectrum antibiotics, such as Metronidazole, Clindamycin, Tinidazole, and Secnidazole, and the infection usually clears up in about seven days (*Diagnosis and Treatment - Mayo Clinic*, 2021). Decreased antibiotic susceptibility is associated with *Gardnerella* in the biofilm state, meaning it does not respond to antibiotics very well. BV's decreased antibiotic susceptibility leads to high recurrence rates, so many females have to get treated multiple times. Although BV is a common and treatable condition, it can increase the risk of contracting a sexually transmitted disease (STD) and increase the risk for pelvic inflammatory disease (PID). Both STDs and PID can cause another set of problems, one of them leading to infertility. A better treatment option is necessary due to its prevalence and recurrence rates.

Gardnerella in the biofilm state is when the cells are enclosed in an extracellular polymeric substance (EPS) matrix. The EPS that makes up part of the biofilm is a mix of polysaccharides, or carbohydrates, and proteins. Because polysaccharides provide the most strength to the biofilm, we will be focusing solely on how they affect biofilm formation. To address this problem, we will identify the primary polysaccharide components of the *Gardnerella* biofilm by utilizing staining and microscopy techniques; these techniques allow for visualization. The polysaccharides of interest will then be visualized and quantified using fluorescence microscopy; fluorescence microscopy is another imaging technique that allows for detection of a fluorescence signal through the excitation of fluorophores. Once we have identified the dominant carbon sources, the same staining and microscopy techniques will be applied to *Gardnerella* grown in synthetic vaginal media (SVM), and it will be supplemented with those select carbon sources. Next, through staining quantification, another way to allow for visualization, we will understand how carbon sources can modulate *Gardnerella* biofilm synthesis. Finally, based off of these results, we will predict and test *Gardnerella* biofilm disruptors using scanning electron microscopy, which creates an image by scanning a focused stream of electrons over a specific surface. The end goal is that these biofilm disruptors will prevent the biofilm from continuing to form, thus lowering the recurrence rates. For this project, a few constraints are involved. SVM will be used, and all testing will be done *in vitro*; this is because the techniques we plan to use cannot be done *in vivo*. In addition, we are building from the work previously done by Lillian Dillard, a biochemistry PhD candidate.

At the end of the project, we hope to have an improved treatment option for BV that can be made into a medication for patients. Ideally, the medication will be able to be taken along with antibiotics, and patients will only need a prescription from a doctor. It is important for our new treatment option to be targeted so it does not kill the healthy bacteria, safe so it does not cause

complications, simple so patients do not need a doctor's assistance when getting treatment, and cheap so it is easily accessible. After this project is successfully completed, hopefully this research will open the door for better treatment options for other diagnoses that have decreased antibiotic susceptibility.

Investigating the Need for Better Infertility Treatment Options for Women

Is there a need for a more accessible and effective infertility treatment option in the southeast of the United States?

Infertility is defined as the inability to achieve pregnancy after 12 months of appropriately timed intercourse (*How Common Is Infertility? | NICHD - Eunice Kennedy Shriver National Institute of Child Health and Human Development, 2018*). It can be broken down into two different categories: primary infertility and secondary infertility. Primary infertility is when a couple has never been able to conceive, and secondary infertility is the inability of a couple to conceive after previously being successful. Fertility declines more rapidly with age in women, and this can be difficult because a lot of women believe that “motherhood is the ultimate expression of femininity”, and it is seen as a direct reflection on their identity and self-image (Peterson et al., 2007).

When there are issues with ovulation, treatment is usually medication, which includes clomiphene, tamoxifen, metformin, and gonadotrophins (*Treatment for Infertility - NHS, 2017*). If the cause of infertility is related to the fallopian tubes, surgery can be performed to break the scar tissue thus making it easier for eggs to pass through (*Treatment for Infertility - NHS, 2017*). If the cause of infertility is related to the uterus or ovaries, laparoscopic surgery can be performed to destroy the extra growths (*Treatment for Infertility - NHS, 2017*). The last treatment option for infertility is assisted contraception. IUI involves inserting sperm directly into the uterus, and *in*

vitro fertilization (IVF) involves fertilizing an egg outside of the body and inserting it back into the uterus. Even with all of these different treatments, some couples are still unable to get pregnant. In addition, not everyone in the world has equal access to the treatments. Do we need to find a more accessible and effective treatment option in the southeast of the United States?

The Progression of Treatment Options

Before the 1970s, infertility was not looked at as a medical issue, and part of this is due to the fact that menstrual cycles had just been discovered as a good marker of ovulation (Buster, n.d.). In 1978, Robert Edwards and Patrick Steptoe, British physicians, achieved the first successful birth from IVF (Buster, n.d.). Although this was a huge success within the medical community, a lot of religious leaders did not allow the treatment because the baby was made in a laboratory; this was not natural and it was work of the devil (Buster, n.d.). In addition, not every woman in the world has equal access to this treatment and some are not allowed to go through with these treatments. Although there are more treatment options available now, they are still expensive and invasive, making them unappealing to women. It is a lot for a woman to go through, both physically and mentally. For some women it does not help that they also have to jump through extra hurdles to even get the treatment.

Current Access to Treatment Options

IVF is the most common treatment option for women, and for the first embryo transfer, it has a success rate of 41.4% (Shauli, 2021). Similar to normal fertility, IVF success rates also decrease as age increases. 1.5% of live births in the United States are due to IVF treatment, and 4.6% are due to other non-IVF fertility treatments (Pisarska et al., 2019). Although these treatments are widely available in the United States, they are very costly. One round of IVF can cost anywhere from \$15,000 to \$30,000 (Conrad, 2021); some insurance companies will cover

part of the cost. On top of these issues, there has been a 0.370% per year increase in female infertility globally (Sun et al., 2019). This could be an issue because the average age of marriage is also increasing.

Theoretical Framework

Infertility can be seen as a sociotechnical issue because many different medical technologies are used, it is not accessible everywhere around the world, and people view it differently due to their religion, cultural norms, and values.

Methods

In order to investigate this question, I will be looking at where the treatment is available in the southeast compared to more populated areas in the United States, who chooses to go through with treatment and how many people, and is there work being done for better treatment options. In order to figure out who has access to treatment, I will investigate policies of different states and also look at where it is socially and religiously acceptable. In order to see who and how many people choose to get, I will look at different studies published. Lastly, to figure out if there is work being done for a better treatment option, I will look at released preliminary research, if there is any.

Conclusion

From this research I hope to learn more about infertility and better understand if a more effective treatment option for infertility is needed. I hope to understand how different women around the world are affected by it and what routes they choose to take once they are diagnosed. There has been talk about whether infertility is increasing, so this research could be more important than before. I also hope to better understand the *Gardnerella vaginalis* biofilm composition and formation and develop a better treatment option for women with BV. Finding a better treatment

option for BV could significantly improve some women's day to day lives. Also through doing my technical research, I hope to develop new skills within the laboratory and master previous skills. By answering these two questions, it can lead me to also answering the overall question about how we can help progress the treatment options for different diagnoses within women's reproductive health. It is important to address these issues because issues within women's reproductive health can lead to greater health problems.

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