INVESTIGATING PHYTO-BIOACTIVE MOLECULES WITH ANTIFERTILITY PROPERTIES FOR THE PURPOSE OF DESIGNING A MALE CONTRACEPTIVE

An Honors Thesis

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ABSTRACT

Title: **Investigating Phyto-Bioactive Molecules with Anti-Fertility Properties for the Purpose of Designing a Male Contraceptive**
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Reproductive health concerns all matters pertaining to the physical, emotional, and social well-being relating to the reproductive system. Reproductive health is a right, not a privilege. The predominant global male role in family planning is a passive one, where the majority of reproductive responsibility has been assigned to females. This is reflected by the dominant focus of contraceptive research on females, while men do not have access to any form of reversible, long-acting, effective contraceptives. Because of this, men have lost a significant element of their reproductive autonomy. Simultaneously, there is unequal and inadequate distribution of modern contraceptive methods to rural and impoverished communities resulting in the global population epidemic. To address these issues, phyto-constituent bioactive molecules like Abrin, Azadirachtin, and Gossypol that contain antifertility properties will be investigated in order to propose future avenues in male contraceptive research.

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Abstract

Reproductive health concerns all matters pertaining to the physical, emotional, and social well-being relating to the reproductive system. Reproductive health is a right, not a privilege. The predominant global male role in family planning is a passive one, where the majority of reproductive responsibility has been assigned to females. This is reflected by the dominant focus of contraceptive research on females, while men do not have access to any form of reversible, long-acting, effective contraceptives. Because of this, men have lost a significant element of their reproductive autonomy. Simultaneously, there is unequal and inadequate distribution of modern contraceptive methods to rural and impoverished communities resulting in the global population epidemic. To address these issues, phyto-constituent bioactive molecules like Abrin, Azadirachtin, and Gossypol that contain antifertility properties will be investigated in order to propose future avenues in male contraceptive research.

Reproductive Health and The Global Demand for a Male Contraceptive

Reproductive health is an inherent component of the biological well-being and quality of life. As a vital element of the human experience, it has been a subject of medical, societal, and cultural concerns since the dawn of humanity. From Indo-European tribes and Ancient Greeks and Romans to modern Western and Ayurvedic medicine, there has always been a demand for treatments to manage fertility. However, despite this ever present demand, much of the focus of responsibility has been placed on females and discovering active biomolecules for female contraceptives. This approach is ineffective. In many developing regions, modern contraceptive methods do not meet the needs of the local women as they are chemical-based, expensive, and inaccessible to rural communities (Bala & Katare 2016). Instead, nearly 80% of primary health care needs including reproductive health are addressed through herbal contraceptives which can be made easily and widely available to rural communities. However, with the absence of proven herbal contraceptive methods to control reproduction, the developing countries have suffered as their infrastructure, economics, land, and society cannot support the exponentially increasing population (Bala et.al., 2016). In order to address the issue of population growth, there needs to be a revolutionary approach to regulating reproductive health. Rather than forcing women to bear the financial and health-related responsibility, the development of a long-term, reversible male contraceptive will increase the autonomy of both partners in family planning. Implications of this research include targeting the feminization of poverty, the influence of patriarchy in women's health, and unequal access of developing countries to reproductive health resources. To address this issue, non-hormonal,

phytoconstituent-based male contraceptives will be explored with the intent of proposing novel approaches for research.

The world population is increasing by 80 million people per year. In addition, it is estimated that nearly half of all pregnancies in the world are unplanned (Khourdaji et al 2018). Thus, the demand for a male contraceptive extends beyond any individual demographic to include the entire world. Therefore, it is important that contraceptives chosen and developed can meet the environmental and economic needs of millions of people across diverse communities. Due to limited access of many global and national populations to western contraceptives, there has been a growing push to discover and develop traditional herbal methods that can be made available and affordable to even rural communities. The World Health Organization started a program dedicated to the study of traditional medical practices that included a focus on traditional contraceptives to address the population growth epidemic. The goal is to promote contraceptives adaptable enough to meet the needs of people living in developing countries with high populations like India, China, Bangladesh, and several African regions. A long-term, reversible male contraceptive is a domestic issue as well. In the United States alone, it is estimated that unplanned pregnancies cost the country 15 billion dollars a year (Khourdaji et al 2018).

Many propose that the reason behind the significant number of unplanned pregnancies is inadequate access to contraception methods. In addition, “the availability of numerous, effective, and reversible contraceptive choices for women has led to the female partner unequivocally shouldering the primary responsibility for family planning” (Khourdaji et al 2018). The current methods of male contraceptives are condoms and vasectomies which are not effective methods due to each’s limitations--the condom with its high failure rate and vasectomy being often irreversible. The condom is the oldest method of contraception in the United States. Globally, It is used as the only contraceptive method for 5.7% of couples worldwide. In the United States, this percentage is nearly a third of the population. However, the condom, while succeeding at protecting against several sexually transmitted diseases, has a failure rate of 15-18% with general use. With perfect use, the failure rate decreases to 3%. Additionally, most couples do not use condoms as a long-term contraceptive method. Instead, 57% of males in relationships discontinue condom use within the first year Concerning vasectomy, only 2.7% of couples worldwide and 6-13% in the United States rely on this as the primary contraceptive method (Khourdaji et al 2018). However, the usage of vasectomy is often dependent on cultural factors and healthcare accessibility. In addition, statistical likeliness of the return of fertility with vasectomy reversal decreases with the number of years passed since the vasectomy procedure. In one study, for men who had a reversal fifteen years or more after their vasectomy, the rate of pregnancy rates was only thirty percent (Fuchs & Burt 2002). In many developing countries, vasectomy is practiced more frequently. However, as vasectomy is a surgical procedure, potential complications include bleeding, infection, chronic orchialgia,

granuloma formation and recanalization, and potential irreversibility (Khourdaji et al 2018).

Males have very few contraceptive methods, and none that are long-acting, reversible options. Condoms, though they are reversible, are typically only used in casual sexual encounters and are generally abandoned in long-term relationships. In addition, they have a high one-year use fail rate. Despite the more reliable and efficient nature of female contraceptives, of the 1,000,000 contraceptives occurring daily, 50% are reported as unplanned. Within the environment of governments desperately attempting to control the increasing population as traditional gender roles and social statuses are being challenged, it becomes necessary to address the development of a male contraceptive pill (Wersch et al 2012). Rather than forcing women to bear the financial and health-related responsibility, the development of a long-term, reversible male contraceptive will increase the autonomy of both partners in family planning. Implications of this research include targeting the feminization of poverty, the influence of patriarchy in women's health, and unequal access of developing countries to reproductive health resources.

Institutional Ideologies and Influences in the Male Role of Family Planning

Reproductive health emcompasses the biological, social, and cultural factors that define each gender's fertility and ability to negotiate related issues. Reproductive rights are the social construct that facilitates the possession of reproductive health. Some of the issues that concern reproductive health include: "the ability to enjoy satisfying sexual relations without fear of infection, pregnancy, coercion or violence; the ability to regulate fertility without unpleasant or dangerous side effects, and in a manner and to a degree of one's own choosing; and the ability to bear and raise healthy children." (Humble 2003). Established power dynamics, access to reproductive health resources, and education threaten each gender's reproductive health. These threats inevitably result in the high failure rate of current contraceptive usage and the exponentially increasing global population as a result of inefficient contraceptive usage. National government policies control what contraceptive methods to which both genders have access. However, as a woman's reproductive rights, her ability to manage her own body and fertility, threaten the existing patriarchy, these governmental policies often limit women's reproductive rights. Governmental state and national policies, religion, male dominance in the household, and private corporations all threaten female reproductive rights. Thus, the social construct must be challenged. In a revolutionary way, to challenge the male patriarchy construct, the introduction of novel, reversible male birth control methods would serve as the foundation for this social change (Humble 2003).

The Ethical Dilemma and Purpose of a Male Contraceptive

There is a twofold argument regarding the ethics of a male contraceptive. The first investigates male contraceptive in terms of its proposed autonomy in the debate on reproductive planning. The second analyzes the risks and benefits of male contraceptive from a medicinal, pharmacological approach.

The absence of a long-acting male contraceptive threatens male reproductive autonomy. Because men do not have to deal with such issues, their autonomy and bodily freedom is made superior than females. However, because of the absence of a long-acting, reversible male contraceptive, men do not have a choice. They must rely on their partners for contraceptive use. By default, the dependence on women causes men to have lower reproductive autonomy than their female counterpart. If a pregnancy does occur, men often do not have the power to control what happens with the pregnancy, whether it is terminated or not. While these issues are important and directly concern women, it puts men at a default in the reproductive health conversation. While it would be unethical to give over power of the woman's body to the male in terms of pregnancy, the development of such long-acting, reversible male contraceptives would give men their own reproductive autonomy (Campo-Engelstein 2010).

The second, and some would argue more significant, ethical dilemma in regards to a male contraceptive is the level of acceptable risks. While many of the side effects of female contraceptives are accepted as the risks of an unplanned pregnancy outweigh them, male contraceptive pills do not have this option. For men, biologically speaking, their options are no risks (not taking a male contraceptive) or risk (even if there is very little risk involved in taking a male contraceptive). This factor is responsible for the few available male contraceptive options on the market currently. Thus, when it concerns the ethics of a male contraceptive on the market, the risks and benefits of male hormonal contraceptives must be understood. Concerning hypogonadol men on testosterone contraceptive therapy, they had increased lean body mass and decreased fat mass. Healthy men had similar effects from the treatment. However, a clinical trial looking at the effects of using testosterone in combination with progestin agent levonorgestrel found that a side effect of those using the levonorgestrel had an increase in abdominal fat mass. In hypogonadal men, testosterone therapy increased their bone mineral density for up to sixteen years. While no specific side effects have been noted due to the variability among the treatments, some recorded ones include acne, mood changes, changes in libido, night sweats, and an increase in body weight (Wersch et al 2012).

It should be noted that all of these side effects have in the past been reported for many female contraceptives and have not impeded their addition to the market. Thus, when approaching the issue of fear of side-effects, some researchers state that there should be a shared-risk model, in which the benefits and risks to both men and women regarding male contraceptive pill usage should be considered. After all, men have just as

much legal responsibility to the child as the female, thus, it cannot be argued that he would not suffer risk in terms of an unplanned pregnancy. In essence, the couple as a whole would be evaluated in terms of affected health, financial stability, and overall life opportunity and the potential consequences of an unplanned pregnancy. This environment proposes an ethically-gender fair approach in assigning the risks and benefits of contraceptive usage.

Patriarchal Influence in Current Sexual Health Practices and Future Research in Male Contraceptives

Patriarchal societies promote the control and subordination of women. Patriarchy can also be defined in terms of the relationship between both genders such as the expectations of each sex and their treatment and opportunities in the society. Historically, "in ancient societies, the father of the family had absolute rights over the members of the family…he owned not only the house, the lands, the animals, and the slaves, but also the wife, the concubines, and the children" (Campbell et al 2009). Until as recently as the early 1900s, this concept applied to the larger majority of the world's populations. Wives' place in the family was to be subordinate to the husband, who often in many regions outside of Northwestern Europe and North America, was parentally-arranged. Patriarchy is a form of power that allows for male domination while simultaneously controlling, oppressing, and exploiting the female sex. This oppression can be seen through sexual and social mechanisms. The trade off for women is that in such societies, they generally receive protection, love, honor, and a name in society. The main instrument used to control women and oppress them to male power is their reproductive abilities (Campbell et al 2009).

Patriarchy can also be defined in terms of the relationship between both genders such as the expectations of each sex and their treatment and opportunities in the society. It is a form of power that allows for male domination while simultaneously controlling, oppressing, and exploiting the female sex. This oppression can be seen through sexual and social mechanisms. Reproductive liberty opposes these male-dominant, traditional values. For instance, " in many developing countries males often dominate in making important decisions in the family, including those concerned with reproduction, family size, and contraceptive use [including a large responsibility of] the… ill reproductive health suffered by their female partners"(Abiona et al 2010). Socially-promoted norms like male dominance, especially in terms of their physical, social, and often sexual advantage, makes women vulnerable to unwanted/risky pregnancies, sexual exploitation, and sexually-transmitted diseases as they often are unable to negotiate for safer sex practices.

However, as women's expectations of marriage and their place in society, including the modern aspiring career woman, these traditional power roles are challenged, and oftentimes, are defeated. It becomes necessary to address the disparity

between gender relationships to promote equality between both male and female. It is within this social environment that men and women can freely communicate about personal desires and achieve better sexual relationships and health. Success has been found in many traditional societies by facilitating change in values via the current social construct. For instance, the Community Aid and Sponsorship Program and Foster Parents Plan International (CASP-PLAN) program in Delhi uses the tradition of men possessing decision-making responsibility in the family hierarchy by integrating men in the domestic sphere to help raise the children and assist their wives. While not necessarily a matter of reproductive health, inserting the male into the domestic sphere opens his awareness to the challenges women face each day. Upon this perspective, it becomes easier to negotiate reproductive health matters such as male birth control. An additional example that places men as being responsible for contraception usage is from the Philippines. AIDS prevention programs will ask men why they are working, knowing the general answer will be something related to wanting to provide for their families. Taking this central value, they educate the men by saying that if they get AIDS, usually through unsafe sexual engagement, then they will not be able to provide for their families as efficiently. By making the conversation of reproductive health relatable to men, it becomes easier to approach these important issues (Raju 2001).

The use of existing social constructs is the foundation of addressing the issue of reproductive health. However, to create social change, there must be the development and implementation of new constructs. Education to the youth most efficiently accomplishes this objective. Creating programs that promote equal responsibility and status in relationships of males and females should be done for youth education. Additional suggestions that relate to this specific issue include educating newly-weds, a moment where the family power dynamic may not have been established, and thus, suggestions are more likely to have an effect. In India, The SEWA-Rural organization provides new couples with reproductive health resources like birth control and educates them on their proper use. In order for them to be successful in their goal, knowing that often in this culture women do not have much freedom for negotiating their reproductive health, they often will reach out to men prior to the marriage to build a rapport. Additional groups in Delhi work to promote better, more approachable communication between husband and wife about reproductive health issues. These programs are efficient because they use the current social construct to challenge the status quo in order to generate new constructs (Raju 2001). The development of a male contraceptive would interrupt traditional female burden of controlling fertility and open discussion challenging traditional gender roles and patriarchal, social constructs.

Finally, the issue of patriarchy has both global and national implications. Even now, subconscious patriarchal ideologies influence the current contraceptive research even in Western, in gender-equalized societies like the United states. Male patriarchy and gender norms limit the introduction of these methods to the market. One of the key arguments to why there is a female birth control pill and not a male pill is that a

"women's bodies are more simplistic and closer to nature…women's bodies are more controllable and better suited for medical intervention, especially reproductive intervention" (Campo-Englestein 2010). From the high success rate of the many male contraceptives in clinical trials and diversity of potential options, this clearly is misleading. Rather, the discipline of science, whether consciously or not, has been influenced by patriarchal values to control the female body, and upon accomplishing this goal, abandon intention to pursue male contraceptives. This is reflected by there being exponentially more money allocated to female contraceptive research compared to men. In the 1990s, the allocation of money and research resources for contraception was as follows: 60% to high-tech female methods, 3 % to female barrier methods, spermicides, and natural fertility control methods, 7% to male methods, and 30% to multiple methods, mostly directed towards females. Today, many researchers focusing on male contraceptives are unable to perform clinical trials because no pharmaceutical company will provide the necessary funds as they do not see a male contraceptive being a lucrative investment (Campo-Engelstein 2010). Until patriarchal influence is addressed and challenged, the hostile social environment will suppress revolutionary progress in reproductive health and autonomy.

Culture and Socioeconomics in Regulation of Sexual Health and Male Attitude

Culture and socioeconomics of a region provide a foundation to understand the dominant agents in the community's sexual practices and attitudes. For instance, Nigeria has a high population growth marked by its high birth rate. A woman is on average likely to have 5.7 births as of 2008 (Abiona et al 2010). Factors that have been proposed to contribute to this high fertility rate include poor accessibility to contraceptive and family planning resources, the low status of women in society, the high illiteracy rate of the female population in Nigeria, the patriarchal nature of the society, and the absence of male contribution to family planning. In addition, in Nigerian culture, a woman's worth in the society is her domestic capacity and motherhood. Further, the more children a man has, the higher his social status becomes. Thus, it is clear that in such a society, the implementation of a male contraceptive would be largely rejected as it does not fit their perceived needs based on their cultural indoctrination. In the United States, this concerns the cultural propensity of religion in health care and politics. These cultural and religious factors will affect family size and use of contraceptives by couples. For instance, Christian ideology on human sexuality promotes sex as an instrument for procreation. Naturally, then, as contraceptive methods are used to prevent this, religion can disrupt contraceptive use and reproductive health. Thus, when implementing strategies to promote widespread

usage of contraceptives, concerning both genders, an understanding of and respect towards the cultural attitude must be considered.

Culture is relevant in the male contraceptive conversation as it dictates individual group preferences for methods of administration. Scottish males preferred an oral method while Chinese men voted the oral method to be the least favored method. South-East Asia or Indian men stated that a long-term injection method would be most preferable. Australian men chose the oral option. Culture perhaps explains this. In Western cultures, injections are seen as "a painful and frightful bodily intrusion and internal violation. In the East…injections have positive connotation as representing the technological expertise of the West and are perceived as a powerful tool for delivering drugs into the bloodstream, in turn making a person stronger" (Wersch & Eberhardt 2012). Concerning the United States, the most preferable method is a birth control pill, which reflects the currently most used female method. The greatest concern of men when it comes to male contraceptive usage is the potential of side effects and convenience of use.

Finally, a consideration of the socioeconomic status and its influence in the availability and accessibility of contraceptives must be acknowledged. Men of lower socioeconomic status will be at a disadvantage for obtaining contraceptive resources be it a lack of nearby infrastructure like health clinics or an inability to obtain health insurance that covers the resources. However, there are ways to equalize this situation for all socioeconomic classes. Men from neighborhoods with high poverty rates, even despite high sexual experience, had high condom usage compared to more affluent men (Brown & Eisenberg 1995). The implications of this include a deeper and more substantial realization of the personal consequences of an unplanned pregnancy as well as reflects the importance of a widely, cheaply available option of contraceptive. Currently, condoms can be purchased at nearly any grocery store or gas station. Thus, there are fewer obstacles barring people from using these resources. This factor must be considered with the future integration of more male contraceptive forms.

Integration of Education in Changing Gender Roles in Sexual Relationships

Education influences the sexual health and practices of a society. Currently, with the promotion of abstinence-only sex education programs targeted at youth, there is a disparity between scientific knowledge and the awareness of adults, including adolescents. Generally, people obtain their knowledge about the risks/benefits, proper usage, and ways of accessing them from friends, family, social media, health professions, and educational programs. Studies have provided evidence that states a significant amount of Americans are misinformed about the risks and benefits of contraceptive methods. For instance, the potential side effects of oral contraceptives are

often greatly exaggerated by American women. In another study looking at sexually active teenage women, they found that nearly all of them had underestimated their capacity to become pregnant with no contraceptive usage and were not aware of the effectiveness of each contraceptive method. This signifies that the sex education programs that are being offered through academic institutions are insufficient. This suggests that many professionals in charge of these programs either are afraid to provide the real data for fear of backlash from governmental agencies and parents. In 1980, a sample study looking at low-income African American adolescents found that these individuals believed that an IUD could get lost in the body and potentially end up a part of a developing fetus. The issue of this is that if proper education is never offered, then these ignorant youth become the adults of the society, responsible for passing down awareness to younger generations. This was proven when several studies examining the relationship between education, cognitive functioning, and unintended pregnancies found that increased education was clearly correlated to better contraceptive use and usage of more efficient contraceptive methods, Regarding school education programs, 47 states have laws that mandate or recommend sex education programs in the school. The required curriculum includes abstinence messages and positive statements about human sexuality, body image, reproductive anatomy, puberty, decision-making skills, STDS, sexual abuse, AIDS, and gender roles. However, the problem exists because few programs address contraceptive usage, including the proper ways to use each method, the benefits/risks of each method, and how to properly obtain such resources. By promoting abstinence as the primary value, it allows for the youth to be vulnerable to their own ignorance (Brown & Eisenberg 1995).

In addition, implementation of revolutionary educational programs would be necessary in constructing the social environment necessary for the widespread use of a male contraceptive. Education promotes the societal gender roles as they concern sexual encounters and male responsibility in contraceptive usage. With the extensive usage of the female birth control pill, family planning and the unintended consequences have been shifted towards female responsibility. The implication of this scenario is that the male partner becomes a passive partner in the reproductive conversation as well as maintains little to no ramifications of his sexual behavior. However, when it comes to child rearing and regulating reproductive health, there is clear responsibility of the male partner. Thus, radical sex education programs must emphasize the male's obligations and role in sexual relationships. Education of youth, who have not established pre-existing gender and social constructs, is the foundation of addressing social acceptance for men to use long-acting male contraceptives and be a part of family planning.Male responsibility in contraceptive usage should be taught in order to promote social acceptability. Comprehensive sex education programs that equally address both male's and female's responsibility and role in relationships/intimite moments should be taught at early ages in the school.

> "Boys should be told that becoming contraceptive responsible extends beyond just wearing the condom. It includes a desire to share the expenses of contraceptives used by the girl, help and support her to decide the best contraceptive, or postpone sexual intercourse when no contraceptive is available. Both need to be educated in contraception and encouraged to support each other's effort to prevent pregnancy." (Chng et al 2018).

Through education, it becomes possible to address current gender roles in their relation to sexual intimacy and responsibility. Upon this foundation, an environment in which both genders are accepting of their individual responsibility for using contraceptives is established.

Healthcare and Access to Reproductive Health Resources

The responsibility of healthcare cannot be understated in addressing the role of men in pregnancy prevention and family planning. In the United States, there is a disparity between the ideological perspective of male involvement in contraceptive use and the reality of sexual health practices. Young men advocate for contraceptive usage as shown by their own opinions and willingness to motivate their friends to use contraceptives. However, only an estimated 45% of young, American men are committed to preventing pregnancy. There are external factors that influence their contraceptive usage and role in family planning. Relationship status influences young men's contraceptive usage. In casual encounters, men are more likely to be concerned about sexually transmitted diseases and unwanted potential pregnancies; as a result, they are more likely to use a condom. However, as shown from numerous studies, condom usage declines as a direct inverse to the increasing length of the relationship. Male partners explain this change in behavior as trusting the female partner to control the family planning as well as stating that condom usage in serious, long-term relationships could be indicative of a cheating spouse and lack of trust in one's partner. There are two opposing viewpoints on a young male's role in pregnancy prevention. Some feel it is solely the female's responsibility in a relationship: "when you're in a relationship with someone I guess the expectation is then obviously that the girl will be on the pill or more on to the pill…I think it does become a girl's responsibility in a relationship" (Vargas et al 2017). The other perspective is that family planning is the equal responsibility of both genders. This dichotomy reflects a cultural disparity between the growing modern gender role of men and their traditional counterpart. Sex and reproductive education programs can address and change this disparity through increased focus on gender roles in sexual intimacy and relationships. Research has shown that education and support from parents and healthcare providers who emphasize males' responsibility in family planning promotes men to use contraceptives (Vargas et al 2017). After all, the majority of men care for their partner and desire to

foster their health. Yet, due to the prevalence of the female birth control pill and the cultural shift towards female responsibility of family planning, males have been conditioned to be passive participants in contraception practices. Through medicine and reformed cultural constructs, men will be initiated into this conversation. Thus, through creating a community where male contraceptive usage is encouraged, acceptable, and widely available, responsibility of family planning will be equalized across genders.

Healthcare systems and the health insurance people have access to dictate the usage of contraceptives. Currently, vasectomy and condom coverage were left out of the Affordable Care Act's contraceptive coverage which unfairly hurts low-income families. However, the Affordable Care Act requires most private health plans in the United States to cover the full range of contraceptive methods and services for women without copayments, deductibles, or other out-of-pocket costs. It forces women to take reproductive responsibility which can unintentionally punish women for their reproductive abilities. It promotes the feminization of poverty and unequally punishes women for unwanted pregnancies (Sonfield 2015).

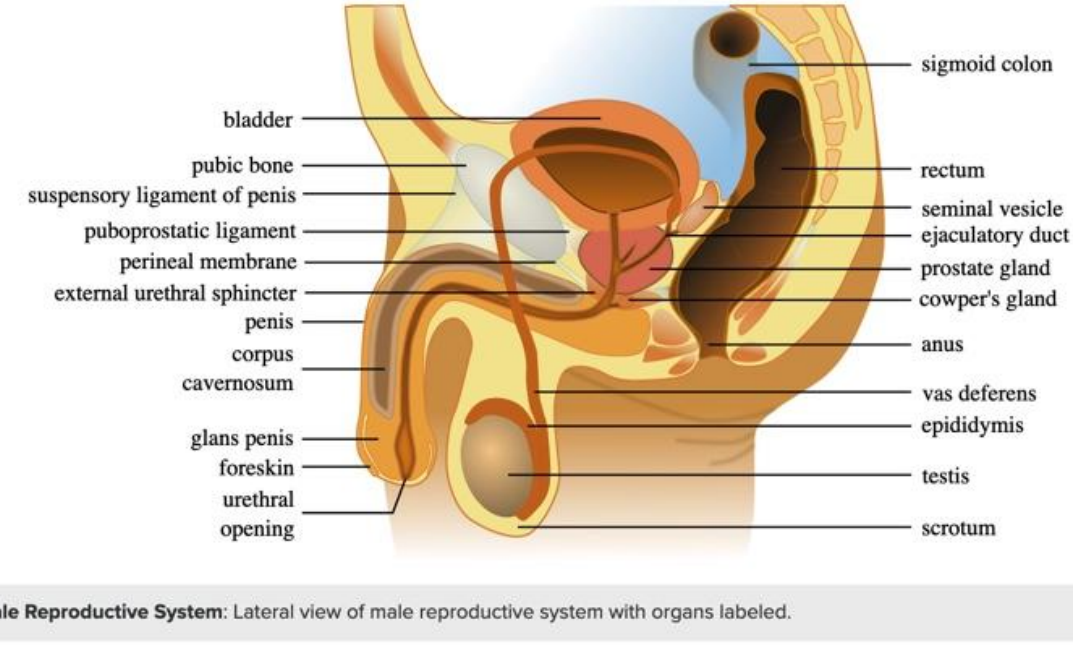
In part, this action of the Affordable Care Act and other health insurance companies was in response to the historical neglect in addressing women's health needs. However, with the knowledge of the current situation with female contraceptives and the global population epidemic, in order to create a society where men have equal reproductive autonomy, healthcare and insurance companies must recognize the need for male contraceptives. Prevention and planning in relation to pregnancies help decrease possible illness, injury, and death to both mother and child. This is only possible by the widespread, easy availability of contraceptive resources. With many females suffering conditions that are contraindications of birth control. Thus, their individual health concerns limit their reproductive control. In order to fill this disparity as well as increase the reproductive autonomy of males, there is a health demand for male contraceptives (Sonfield 2015).

With current research and clinical trials studying the efficiency and safety of male contraceptives, there is no doubt that such medicinal technology is in the near future. However, in order that these reproductive resources are made accessible for the wider public, there must be support of the general healthcare institution and professionals. Like female contraceptives, male contraceptives could potentially have side effects and unintentional drug interactions. Further, male contraceptive methods like oral pill, dermatological methods, and long-term devices will all require prescriptions from medical professionals in order to ensure the health of the male. Because of this, individuals will have their own personal set of needs and risks factors. Proper education, research, training on the part of healthcare professionals will be necessary in order to confront these issues and design efficient treatment plans for patients. By recognizing and implementing policy that equally favors both genders' reproductive usage, reproductive health and global consequences of population growth will be addressed. In

this environment, it becomes possible to advance the conversation on sexual health and reproductive autonomy.

Foundational Anatomy and Physiology of the Male Reproductive System

Anatomy

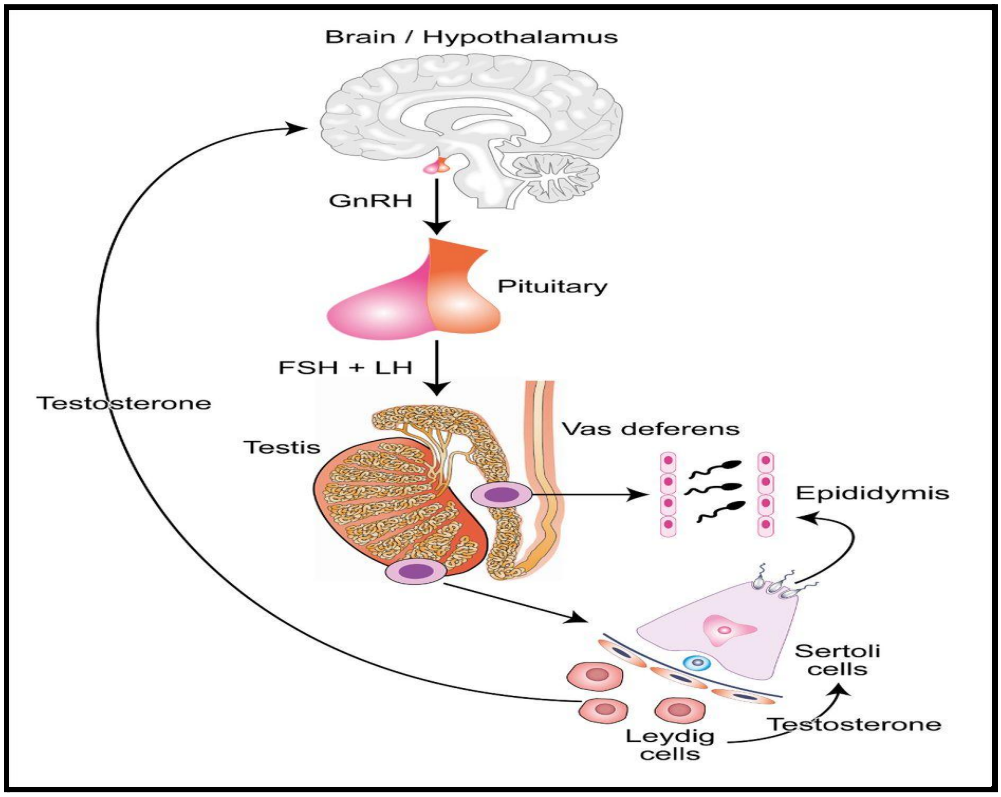


Male Reproductive System: Lateral view of male reproductive system with organs labeled.

The external male reproductive organs include the penis, scrotum, epididymis, and testes. The internal organs, also referred to as the accessory organs, include the vas deferens, seminal vesicles, prostate gland, and bulbourethral glands. Semen is the male secreted fluid that contains the spermatozoa, proteolytic and additional enzymes, and fructose that allows for spermatozoa survival. Spermatogenesis is the physiological process and sequence of structural changes that results in sperm production and occurs in the seminiferous tubules that are located in the testes. The scrotum provides the necessary environment for sperm survival and function. The epididymis is located in the posterior of the scrotum and connects to the vas deferens. It stores and transports sperm. The testes is the location for sperm production. There is a collection of coiled tubes in the testes called the seminiferous tubules. Within these tubes, spermatogenesis occurs. The vas deferens is responsible for transporting the mature sperm to the urethra for ejaculation. The seminal vesicles are pouch-like structures attached to the vas deferens and secrete the fluid element of the ejaculation and molecules like fructose to

give nutrients to the sperm. The prostate gland also provides some of the fluid and nourishment for sperm. The bulbourethral glands are small glands located on the urethra below the prostate gland that secrete a clear fluid that lubricates the urethra and neutralizes the acidity from residual urine ("Boundless Anatomy and Physiology: The Reproductive System" 2021).

Physiology of Spermatogenesis and Male Fertility



Spermatogenesis is the process that results in the production of sperm. It begins in the brain where the hypothalamus secretes gonadotropin-releasing hormone which stimulates the anterior pituitary gland to secrete luteinising hormone and follicle stimulating hormone. These two hormones act on the Leydig and Sertoli cells in the testis, where sperm production takes place. The Leydig cells produce testosterone, which is then secreted into the bloodstream. Testosterone in the blood is important in the negative feedback loop to regulate the production of GnRH and gonadotropins. However, in the testicles, testosterone levels are 40 times higher and facilitate the Sertoli cells in the role in spermatogenesis (Reynolds-Wright & Anderson 2019). Spermatozoa development is dependent on the structural and nutritional support of somatic Sertoli cells. Large, epithelial columnar cells, they are connected to the

seminiferous tubules' basement membrane. Hormones such as androgens and FSH act on the receptors found within the Sertoli cells stimulating and facilitating spermatogenesis. These Sertoli cells are responsible for maintaining an environment suitable for sperm production through secretion of paracrine factors and expressing cell surface receptors. The number of Sertoli cells determines the ultimate spermatozoa production. Sertoli cells cannot support spermatogenesis until they completely mature during puberty (Kretser et al 2017). From the Sertoli cells, sperm are released into the seminiferous tubules and travel through these into the epididymis where they are stored, concentrated, and allowed to mature. During ejaculation, sperm are transported from the epididymis via the vas deferens to the urethra and out of the body (Reynolds-Wright & Anderson 2019). Spermatogenesis takes an average of 64 days in a human. Though the three stages inter-cross each other, they end with the release of mature spermatozoa into the opening of the seminiferous tubules. As each cycle ends, there creates a constant pulse of sperm movement. This factor allows the testes to continually produce sperm so that a normal man is able to produce approximately 1000 sperm per heartbeat (Kretser et al 2017).

There is specific cell and enzyme signaling involving PP1y2, GSSK3, and PP2B along with protein kinase A that are important in regulating the physiological process of sperm maturation and fertilization (Dey et al 2019). Within mammals, male spermatozoa acquire their motility and fertilizing ability during their passage through the epididymis. Post ejeculation of spermatozoa into the female reproductive tract, sperm undergo capacitation, a physiological change that involves structural modifications and allows sperm to penetrate and fertilize an egg, and hyperactivation. Some of the changes that occur during capacitation include the removal of the plasma membrane cholesterol resulting in a decreased phospholipid to cholesterol ratio, plasma membrane hyperpolarization, an increase in intra-sperm pH and cAMP levels, and increase in calcium uptake, and an increase in tyrosine phosphorylation thought to be due to an increase in protein kinase A activity. These biochemical changes during capacitation result in a sperm being able to penetrate the oocyte's extracellular matrix, the zona pellucida (a thick transparent membrane that surrounds the oocyte and assists with implantation), and complete the acrosome reaction to achieve fertilization. In addition, capacitation occurs almost simultaneously as hyperactivation, which is a process that results in increased functioning of the flagellum to give it a strong whiplash motion for motility in the female reproductive tract and allow for it to fuse with the oocyte. Although it is known that sperm maturation and initiation of motility involve and are regulated by changes in metabolism, cyclic adenosine monophosphate signaling protein, calcium interaction, and pH via protein kinases and phosphatases, the exact biochemistry and physiological pathway that allows sperm to acquire their motility in the epididymis and the cellular pathway that results in a varied motility necessary for moving through the female reproductive pathway is unknown. The article's review suggests that the three signaling enzymes PP1y2, GSSK3, and PP2B along with protein

kinase A are important in regulating the physiological process of sperm maturation and fertilization. One important phosphatase enzyme is PP1y2, present in the testis and sperm of mammals, and is believed to regulate normal function of mammalian sperm. There are three protein molecules that regulate the biochemical activity of PP1y2: inhibitor 2, inhibitor 3, and SDS22. Changes in the phosphorylation of these three molecules influence the conformation of these molecules and the activity of PP1y2 to allow for regulation of the processes involved in initiation and activation of sperm motility. One of the protein kinases involved in this phosphorylation is GSK3 which is an enzyme involved in epididymal sperm maturation and fertility. Calcium cell signaling is also thought to influence initiation of motility as there are decreased levels of calcium during this period of time. This suggests that sperm phosphatase (PP2B) activity, which is activated by calcium, is decreased during epididymal sperm maturation and initiation of motility (Dey et al 2019).

Spermatozoa in the testicles are nonmotile and infertile. However, during the crossing of the epididymis, spermatozoa undergoes motility initiation and acquires fertilization capacity. Some of the specific events during motility initiation are the activation of sperm flagellar activity and ciliary motility. This activity is regulated by cAMP, calcium, and pH. cAMP and protein kinase A are involved in regulating sperm function. Adenylyl cyclase uses ATP to synthesize cAMP in sperm (Dey et al 2019). Phosphodiesterase enzymes including PDE1, PDE4, PDE8, PDE10, and PDE11 degrade cAMP when cellular cAMP levels must be decreased. cAMP functions through protein kinase A. Inhibition of adenylyl cyclase or protein kinase A results in defective sperm motility and inability of sperm to undergo hyperactivation. pH and calcium are involved in regulating sperm function. Increased intracellular pH activates motility of sperm. Recent studies suggest that the increased intracellular pH initiates calcium influx required for hyperactivation. There are special calcium channels that are necessary for calcium influx in sperm called CatSper. When sperm cytosol was alkalinized followed by activated the CatSper channels, hyperactivation of the sperm was achieved (Dey et al 2019).

Finally, protein phosphatase PP1y2 is an enzyme involved in sperm function. There are four isozymes of this enzyme that are all encoded by three genes. They vary based on the structure of their c-terminus. High concentrations of PP1y2 correspond to low sperm motility while low concentrations of the phosphatase correspond to high sperm motility. However, a loss of this enzyme results in impaired spermatogenesis and a reduction in epididymal sperm count. Thus, it is believed that the enzyme plays two roles, one during spermatogenesis and the other on sperm after they leave the seminiferous tubules. PP1y2 and serine/threonine protein kinases regulate the necessary phosphorylation patterns during spermatogenesis. Additional information regarding the biochemistry of this enzyme and its role in sperm maturation and initiation of motility are still being researched (Dey et al 2019).

Male Contraceptive Research: Hormonal and Non-Hormonal Approaches

The history for the developing novel forms of male contraceptives beyond the condom and vasectomy started in 1965 with the development of WHO Special Programme of Research, Development, and Research Training in Human Reproduction. The organization searched for new modalities for male contraceptives. They developed the no-scalpel vasectomy, now the globally-accepted method. In addition, they looked at the potential of testosterone-derived oral and injected male contraceptives. They found that 70% of men became azoospermic when administered 200 mg/ week of testosterone. In the trial testing this, in the year that couples used this as their only source of contraceptives, only 0.8 pregnancies per 100 people occurred. In the 1990s, there was a great deal of research looking at the role of progestogens and reported success in terms of spermatogenic suppression. In the UK and in Australia, they were exploring the potential of long-acting reversible contraceptives such as testosterone pellets combined with etonogestrel implants and depot medroxyprogesterone acetate. In a 35.5 year range, there was not a single pregnancy in the fifty-five couple subjects. In China, there were large studies looking at long-acting testosterone undecanoate with norethisterone enanthate. The study has 300 couples participate, and 96.8% of the men in the study had a spermatogenic suppression that lasted the entire year duration of the study. Of the 300 couples, there were only 4 pregnancies meaning the approach had only a 1.59% fail rate, which is less than most female contraceptives. Currently in clinical trials are several hormonal approaches that target GnRH, FSH, or LH hormones. One is the nestorone-testosterone gel which has entered the international phase IIb. It suppresses the production of FSH and LH to prevent production of sperm. In terms of developing non-hormonal approaches to act on spermatogenesis, methods such as bisdichloroacetyldiamines (which prevents the conversion of vitamin A to retinoic acid, an essential compound for spermatogenesis in the testis), bromodomain testis-specific protein inhibitors (which would inhibit the expression of necessary proteins for spermatozoa maturation), lonidamine derivatives (a non-hormonal drug that has antispermatogenic properties), and thermal treatment (using an increase in testicular temperature such as taking a hot bath or modifying one's underwear to promote a natural reduction in sperm production) (Reynolds-Wright & Anderson 2019).

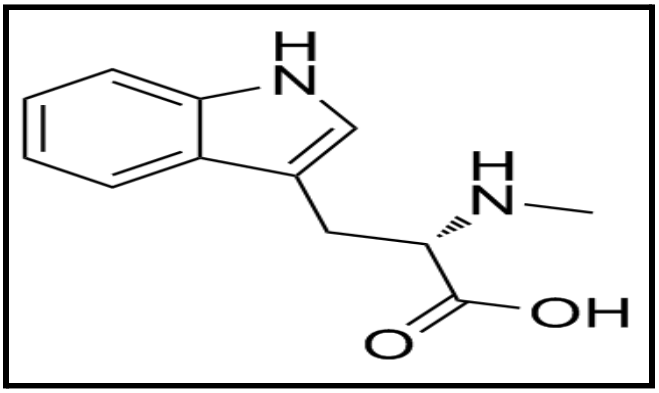
Hormone-based contraceptives typically induce infertility by affecting spermatogenesis and producing azoospermia by suppressing the secretion of gonadotropins and intratesticular testosterone (Yapar & Inai 2012). A male hormone contraceptive would have to disrupt the physiological pathway of testosterone production which involves the secretion of hormones from the hypothalamus and pituitary that activate the testes to produce testosterone and sperm. The hypothalamus first secretes gonadotropin-releasing hormone which acts on the anterior pituitary gland stimulating the release of luteinizing hormone (LH) and follicle-stimulating hormone

(FSH). LH binds to the Leydig cells in the testicular interstitium and activates the metabolic pathways to result in the production of the sex hormone testosterone. FSH acts on the Sertoli cells to stimulate spermatogenesis. In the testicles, the testosterone concentration is 100 to 200 times greater than the serum concentration. This allows for spermatogenesis to happen within the seminiferous tubules. Research suggests that a male hormone contraceptive would have the most effectiveness by acting on the negative-feedback circulating testosterone pathway between the hypothalamus and pituitary gland. A down regulation of these two organs in the brain would decrease the amount of LH and FSH, and as a result, suppress spermatogenesis. To maintain this effect in the body, the male hormone contraceptive would need to be introduced in the body for two to three months prior to achieving infertility (Khourdaji et al 2018).

Non-hormonal techniques typically impact sperm function and maturation. The advantage of non-hormonal techniques is that they have a rapid onset in infertility and do not interfere with non-reproductive androgen-dependent function in the body (Yapar et. al. 2012). Bioactive phytoconstituents from *Curcurma longa, Abrus precatorius, Barleria prionitis, Piper nigrum, Capparis aphylla, Bacopa monnieri, momordica charantia*, and hundreds of additional plant species have been studied for their reported antifertility and antiandrogenic properties. However, due to several decades of research and experimental studies demonstrating the strong potential of these bioactive compounds for male contraceptives, the phytoconstituents azadirachtin from *Azadirachta indica* and gossypol from the Gossypium family will be explored.

Phyto-bioactive Constituents with Anti-Fertility Properties

Abrin: *Abrus precatorius*



Abrus precatorius is currently used as an antifertility agent in many middle eastern regions including Pakistan and many south Asian countries (Abbasi et al 2009). *Abrus precatorius*, of the family leguminosae, is native to India subcontinent and the East and West Indies. In Hindi, it is called Ratti or Gumchi. There are bioactive molecules located in the roots, leaves, and seeds of the plant but each location varies in specificity. Abrin is found in the seed. *Abrus precatorius* has been used for its medicinal properties since ancient times as recorded by ancient Chinese texts and historical accounts from other prehistoric cultures (Bhakta & Das 2020). The chemical constituents of *Abrus precatorius* that allow for its medicinal properties include glucoside, abrusic acid, haemagglutinin, poisonous proteins, a fat splitting enzyme, and abrin (Nadkarni 2015). The seeds of *Abrus precatorius* possess the majority of the bioactive molecules with antifertility capacity. The seed contains abrine, abrin A, B, C, I,

II, III, Abrus agglutinin, saponin, flavonoids, abrectorin, precatorin, lectin, and campestanol (Bhakta & Das 2020).

The antifertility properties of *Abrus precatorius* have been tested both from a crude extract involving compounds of the entire plant and the individual compound abrin. Using a crude extract, oral administration of *A. precatorius* in male mice in experimental procedures overall resulted in anatomical modifications including decreased thickness and number of seminiferous tubules, shrinkage of the tissue surrounding the seminiferous tubules, decreased amount of spermatozoa within the lumen of an individual seminiferous tubule, reduced thickness of the fibrous layer surrounding the seminiferous tubules, and reduced amount of sertoli and leydig cells within and between the seminiferous tubules in a single focus. These anatomical modifications are important as they inhibit spermatogenesis. Some studies demonstrate that *A. precatorius* has a reversible antifertility/antispermatogenic effect in mice within 20 days of withdrawal (Bhakta & Das 2020).

Ethanolic extracts prepared from the seeds that were intraperitoneally injected with the doses of 20, 40, and 60 mg/kg in mice produced the previous anatomical modifications. In addition, it interfered with the plasma testosterone levels which disrupted spermatogenesis (Joshi & Aksha 2011).

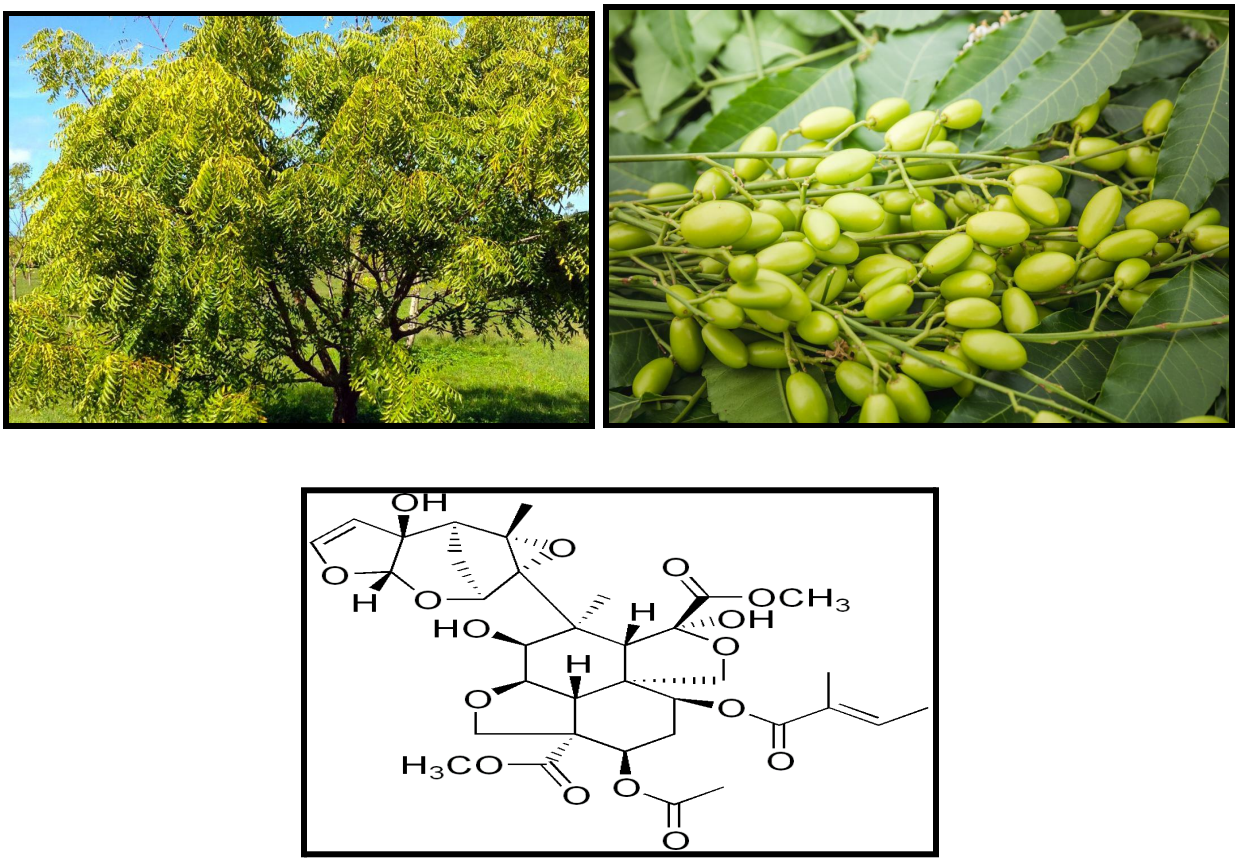
In another experiment to test *Abrus precatorius* on human sperm samples, a crude seed extract was prepared and tested on spermatozoa samples. The extract worked primarily to inhibit the motility of spermatozoa. At a concentration of 1.25 and 2.5 mg/mL, spermatozoa motility was suppressed within 60 minutes after exposure. A 5.0 mg/mL concentration, inhibition of movement was achieved in a half hour. At a concentration of 20.0 mg/mL, within 5 minutes, all samples had immobile sperm (Ratnasooriya et al 1991). The results of this study suggest that bioactive molecules interact with the plasma membrane of sperm resulting in disruption of the integrity of the membrane, subsequent hypoosmotic swelling, and loss of motility.

There are several proposed hypotheses for how A. precatorius interacts in the body to induce temporary infertility. Ratnasooriya et. al proposed two hypothetical theories for how *Abrus precatorius* disrupted structural integrity. The first, based on previous evidence with medicinal drugs that are known to disrupt functional integrity of the plasma membrane, suggests that there is an increased intracellular calcium which is detrimental for sperm motility. The second suggestion is that *Abrus precatorius* interferes with the production of cAMP by upregulating or inducing its own phosphodiesterase activity. Antifertility is facilitated by a reduction in cAMP levels, and if *Abrus precatorius* inhibited adenylyl cyclase activity or otherwise disrupted the production of cAMP, then male infertility would be achieved. A final mechanism by which *Abrus precatorius* works could be the result of one of its compounds abrin, an amino acid derivative, known to generate reactive oxygen species. These RO species could cause lipid peroxidation to the plasma membrane of sperm which would disrupt their structural integrity and inhibit sperm motility (Ratnasooriya et al 1991). Additional

research by Abbasi et al in 2019 suggests that abrin facilitates much of these antifertility properties. The suggested mechanism for the inhibition of fertility includes how abrin inactivates rRNA and prevents the protein synthesis of sertoli and leydig cells. However, others have proposed that it directly interacts with the mitochondrial membrane of spermatids causing apoptosis of spermatids, while the steroids in *A. precatorius* replace the natural steroids of LH, FSH, and testosterone which all prevent spermatogenesis. (Abbasi et al 2019)**.** In addition, Abbasi proposed that "the best possible mechanism for the dose dependent effect of seed extract…is, abrin and agglutinin…can induce apoptosis by interacting with mitochondrial membrane and thus, inactivating mitochondria" (Abbasi et al 2019).

However, due to the nature of *A. precatorius* being toxic, little empirical research has been generated to understand the enzymatic interaction of abrin with human biochemistry and metabolism. Thus, future research regarding design of male contraceptives with *A. precatorius* is impeded.

Azadirachtin: *Azadirachtin indica*



A) Image of Azadirachta indica. Known as the "Tree of Life" in Ayurvedic medicine, it is used in more than 75% of traditional herbal therapies. B) The seeds of Azadirachta indica. The seeds contain the highest source of azadirachtin in the entire tree. Azadirachtin is extracted from suspensions created by crushing the seeds. C) The chemical structure of azadirachtin.

There are twelve closely-related isomers of azadirachtin that collectively are referred to as azadirachtin

Azadirachta indica is commonly known as neem. Neem is able to interact with the spermatogenesis process and alter the structure and function of the testes which therefore inhibit the integrity of the spermatozoa and disrupts the normal functioning of the male reproductive system. Neem is found in several regions including Asia, Africa, America, and Australia. It can survive in different conditions such as rocky and dry soil and in various climates. It grows up to 700 meters in height and lives for more than 200 years. Neem has been used for its antifertility properties in both genders across the globe. For instance, women in northwestern Madagascar villages consume neem leaves for their contraceptive use. In Gambia and Ghana, neem leaves are made as a tea drink to prevent pregnancy. These tea drinks are given to people by the first two or three years of their lives (Seriana et al 2019).In the 1980s, neem oil was first tested as a male contraceptive in monkeys and found to induce reversible infertility without affecting sperm production or libido. In many rural regions of India, men consume neem extract to achieve the contraceptive effects (Bhatt 2016).

The bioactivity of neem is largely due to azadirachtin, a mixture of 12 closely related isomeric meliacins known as Azadirachtin A-G. They are present in the seeds and leaves of the plant. Azadirachtin concentration in neem oil extract can range from 300 ppm to over 2500 ppm depending on the source of the plant the extract was made and the solvent used to extract the azadirachtin (Mishra et al 2013). Seeds have a higher concentration. Nimbin has also been proposed to have spermicidal pharmacological activity. Additional bioactive constituents of the plant include salannin, nimbin, nimbinin, nimbidin, 6-desacetylnimbin, phenolic compounds, carotenoids, steroids, and ketones (Kumar et al 2016).

Neem trees are often referred to as the “Tree of Life" because of their extensive application in traditional medical disciplines like Ayurveda. More than 75% of Ayurvedic remedies contain some part of the neem tree, and due to its widespread consumption and usage, neem has been determined to be safe at extreme dosages. However, such dosages have rarely been tested in a clinical or laboratory setting. A daily dose of 100 mg/kg for twenty days in a human has been known to affect thyroid functioning. Thus, there are limitations to doses, however based on past data using male rats combined with a consideration of the differing physiology in humans, a daily safe dose has been determined to be 0.002 mL/kg body weight (Kumar et al 2016).

The physiology behind neem’s antifertility properties involves interrupting the production of the male sex hormone testosterone. Testosterone is an endocrine hormone that regulates spermatogenesis in the testis. Luteinizing hormones stimulate the Leydig cells to produce testosterone, which acts as a paracrine acting on adjacent

cells in the testicular seminiferous tubules. Testosterone's main regulatory function as a paracrine chemical messenger is to control the proliferation of spermatogonia by guiding spermatocytes to become spermatids. It allows germ cells to develop into spermatozoa during the process of spermatogenesis. Testosterone is able to diffuse and act on nearby target cells by binding to intracellular receptor proteins in the cytoplasm and nucleus. In the testes, the concentration is between 340-2000 nM while the plasma levels are 8.7-35 nM. When there is a reduction of testosterone receptors in animals, there is inhibition of germ cell formation by interrupting the prophase of meiosis during the diplotene and pachytene stages thus preventing sperm differentiation and maturation (Seriana et al 2019). Neem leaves extract also has an impact on the concentration of follicle-stimulating hormones. Follicle-stimulating hormone (FSH) is a family of glycoproteins and their specific receptors, FSH-R proteins, that when activated interact with G proteins. These receptors are composed of seven-transmembrane receptors. Sertoli cells have a receptor specific to FSH, and when FSH binds, Sertoli cells are able to additionally regulate spermatogenesis. If this process does not occur, spermatozoa levels decrease. FSH works by optimizing the proliferation and production of spermatogonia germ cells. FSH works as a transcription factor; when it binds to FSH-R in the Sertoli cell, it activates the genes and therefore proteins needed for survival and differentiation of these spermatogonia germ cells. FSH also is able to regulate the proliferation and differentiation of Sertoli cells. By decreasing the concentration of FSH being produced, spermatogenesis is disrupted, thus decreasing the production of spermatozoa (Seriana et al 2019).

Two different administration techniques have been used to analyse the antifertility properties of azadirachtin in male mice which include oral and peritoneum injection.

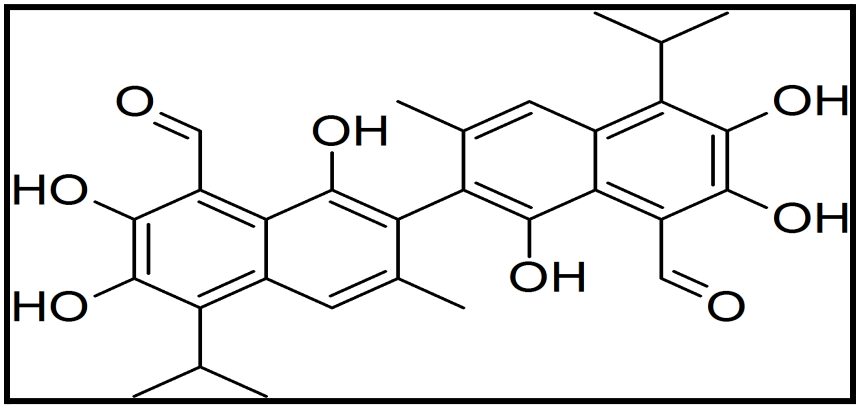
The general observation for oral neem extracts inducing infertility is affecting the histology of the male reproductive system and interrupting spermatogenesis. Studies stated these changes to be reversible. Reported effective oral dosages for inducing male infertility using neem extract have ranged from 50 to 500 mg/kg (Seriana et al 2019). Mishra and Singh demonstrated that an oral dosage of 200 mg/kg of body weight resulted in histological modification in the epididymis and seminiferous tubules which disrupted spermatogenesis and reduced sperm concentration, but was reversible after 42 days of treatment withdrawal (Mishra et. al., 2005). Additional studies have reported that oral dose of 100 mg induced cellular modifications in the Sertoli Cells, Leydig cells, and spermatids which resulted in infertility. For instance, a dose of 100 mg/kg of body weight for ten weeks interfered with spermatogenesis and impacted the integrity and motility of the sperm (Parshad et al 1998). The average time interval for return of fertility is four to six weeks (Seriana et al 2019).

Peritoneal injections have been suggested as a possible long-term male contraceptive. For instance, a small concentration of neem extract would be injected in the vas deferens to induce infertility for several months. A peritoneal injection

consisting of 50 microliters of neem oil into the lumen of the vas deferens in male mice was performed to study the potential long-term contraceptive applications. In the male mice subjected to injection of neem oil, they all remained infertile for an eight month period. In addition, none of the subjects demonstrated an affected libido. A histological analysis was performed. There was no occlusion, granuloma, or inflammatory changes to the vas deferens and epididymis. The lumen of both was patent, and the epithelial cells lining the tubule did not have varied morphology. These histological studies showed that spermatogenesis was impaired as early as two weeks after initiation of treatment. By week four in the male mice, there was a recorded significant reduction in the seminiferous tubule diameter and complete inhibition of spermatogenesis. The Leydig cells did not appear affected. The intra-vas administration of the neem oil resulted in an inhibition of spermatogenesis without affecting testosterone production. Upon further experiment, it was proven that a unilateral administration into a single vas deferens resulted in localized reduction of testicular size and spermatogenic inhibition that did not carry over to the second vas deferens (Dhawan et al 1993). Additional studies focused on the effect of a daily intraperitoneal dosage of 50, 100, and 150 mg/kg of neem extract in male rats for fifteen days reported a reduction in testosterone concentration (Seriana et al 2019).

Concerning toxicity of neem extracts, oral consumption of high doses of azadirachtin can be toxic as it interferes with the generation of the proton gradient during the electron transport system, and as a result, inhibits the aerobic production of metabolic energy. However, most reported cases associated with neem oil poisonings concern children. Generally, toxic doses involve 25 to 60 mL of neem oil, a highly concentrated form. Neem extracts are by far less concentrated than neem oil (Mishra & Dave 2013). Therefore, additional research should be performed to determine the conversion concentration of neem oil to safely induce the same effect of infertility as neem extracts.

Gossypol: Gossypium Cotton plants



A.) Image of Gossypium cotton plants. Gossypol is a natural phenol found in cotton plants B) The cotton kernel. Gossypol is a yellow pigment in the seed that inhibits several dehydrogenase enzymes. The seeds contain the highest source of azadirachtin in the entire tree. Azadirachtin is extracted from suspensions created by crushing the seeds. C) The chemical structure of gossypol. In the body, gossypol tautomerizes to three forms: the aldehyde, hemiacetal, and ketone form.

Gossypol is a yellowish pigment in cotton plants present in the seeds and root bark. Its primary function in nature is to protect the plant against insect pests. It is a highly reactive polyphenolic dialdehyde and is a weak organic acid with a pKa of 7.2 (Porat, 1990). The chemical properties of Gossypol are important in the metabolism of the compound. Edwards et. al found that it has a molecular weight of 518.54 and a structure consisting of 2,2'-binaphthalene-8,8'-dicarboxaldehyde-1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl. In the body, gossypol tautomerizes to three forms: the aldehyde, ketonoid, and hemiacetal forms. In most solvents, gossypol exists in its aldehyde form. It racemizes in the body, which might be due to the atropisomerism, or restriction of two naphthalene units around the interlinking C-C bond, of the molecule (Qian et al 1984). Its tautomerization is important in the biochemical interaction in the body. The aldehyde groups on the

compound can bind to proteins in the body via aldehyde-amino group linkage. The peptide bonds all the compound to be transported through the renal system via the organic anion system. There are two enantiomers of the compound. The elimination half-life of the positive enantiomer is longer than the levotory enantiomer. Research suggests that the -enantiomer could be secreted faster due to its decreased ability to bind to proteins in bodily tissues (Porat 1990).

Its antifertility properties were discovered in 1957, when it was reported that between 1930 and 1940, Wang village in Jiangsu, China had no childbirths. This phenomenon corresponded to a switch from soybean oil to cottonseed oil for cooking due to economic reasons. Based on these reports, scientists hypothesized that the biologically active compound in cottonseed oil must have female antifertility properties as women during this period of time suffered menstrual disturbances. However, Hubei Provincial Group demonstrated crude cottonseed oil to have antispermatogenic effects in rats and monkeys. Later, other studies proved the bioactive molecule was gossypol (Qian et al 1984).

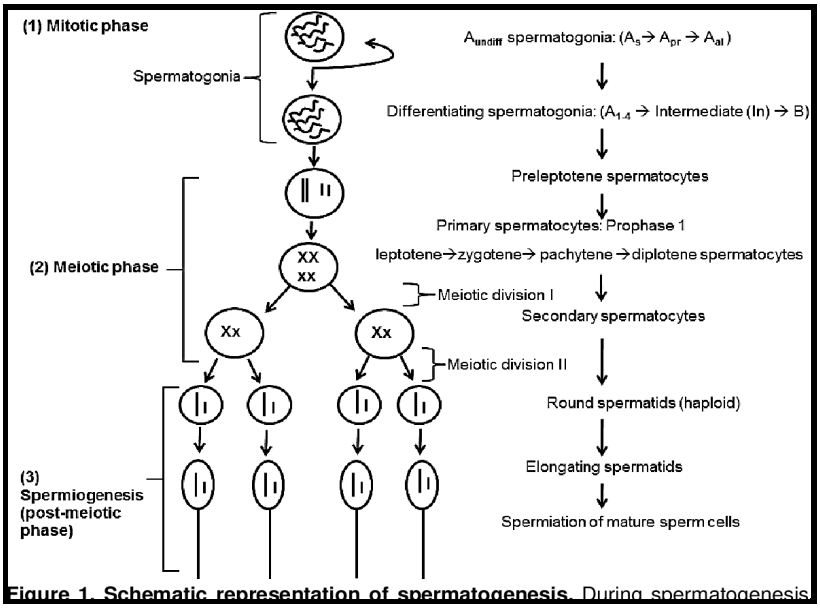


Figure 1. Schematic representation of spermatogenesis. During spermatogenesis

Shown is the spermatogenesis pathway. Gossypol interacts with spermatogenesis to induce infertility. Spermatozoa, spermatids, and spermatocytes of mid to late stages of differentiation are vulnerable to gossypol manipulation.

Gossypol has been suggested to affect the motility of sperm without impacting other male reproductive functions. The sperm motility is a central part in the reproductive process. Spermatogenesis is a continuous process that takes about 70 days to complete in humans. It involves many cycles of cell division, differentiation, and maturation. It is a heavily regulated process, controlled by many hormonal and non-hormonal factors. In the testes, the spermatozoa are immature, immotile, and

incapable of oocytes-fertilization. When the spermatozoa pass through the epididymis, they become mobile and are modified with proteins and other biomolecules to become mature. The sperm are able to move by their flagellum. In mammals, in order for fertilization to happen, the sperm must use its flagellum to enter the female reproductive tract and bind to the oocyte. Surface molecules that are embedded in the plasma membrane of the sperm facilitate their mobility. Thus, any modifications of the plasma membrane can decrease fertility capacity. For instance, using antisperm antibodies that bind to the plasma membrane can prevent sperm locomotion. However, once the sperm has been introduced to the female reproductive tract, it is regulated by the epithelial cells and secretions of the female (Porat 1990).

Gossypol is a high hydrophobic molecule allowing it to interact easily and effectively with the phospholipid bilayer of the biological membrane. Because of this, gossypol is able to affect the properties of the plasma membrane and disrupt the membrane structure, electrostatic charge and separation, and transmembrane ion fluxes. It is also an antioxidant that can inhibit many kinetic and oxidoreductase enzymes in the body meaning it can affect basic metabolism. Spermatozoa, spermatids, and spermatocytes of mid and late stages of differentiation have been suggested to be highly susceptible to gossypol manipulation (Porat 1990).

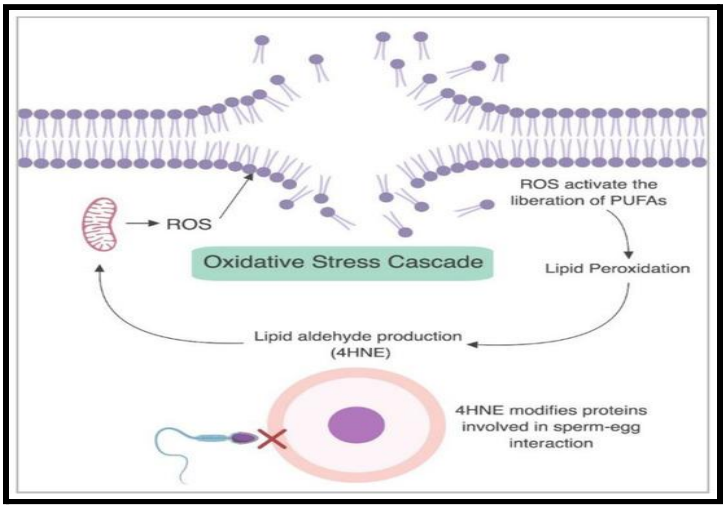
Gossypol is believed to induce infertility by disrupting the integrity of the plasma membrane of spermatozoa, which is responsible for their membrane transport, electrical potential, and survival. It is involved in the acrosome reaction, sperm capacitation, sperm metabolism, and binding of the sperm to the oocyte. Inhibition of the hydrolytic enzyme sperm acrosome could potentially be the most direct method in which gossypol inhibits sperm motility (Porat 1990).

Additional literature states that gossypol is capable of interacting with the Sertoli cell junctions resulting in these cells being unable to regulate and produce a functional blood-testis barrier. The significance of this is that spermatogenesis is associated with cell to cell signaling as the processes of mitosis and differentiation of spermatogonia, meiosis during stage XIV, the transition of preleptotene/leptotene spermatocytes across the blood-brain barrier, spermiogenesis in steps one through nine that involve the differentiation and migration of spermatids across the seminiferous epithelium, and the process of spermiation where mature spermatids are released. Cell-cell interactions regulate these events. Thus, by impacting the cell junctions of Sertoli cells, spermatogenesis is disrupted. The affected cell junctions correspond to interrupted cellular communication. Experimental observations demonstrate gossypol inducing a gap junction uncoupling. The molecular mechanism for this is unknown, but outside research has suggested the decrease in connexin-43, a major gap junction protein found in Sertoli cells, along with plasma membrane proteins ZO-1 and N-cadherin relocating to the cytoplasm, being important in the explanation (Wong et al 2010).

Because of the high success of gossypol as a male antifertility compound, human trials were performed in the 1970s. Doses were based on the accepted standards set by

the FDA which states that the daily dose for gossypol can be up to 450 mg/kg. Internationally, the accepted standard is 600 mg/kg. Quin et al found that a daily dose of 60-70 mg per day for 35 to 42 days resulted in a gradual decrease in motility of spermatozoa, followed by oligospermia, necrospermia, and azoospermia in all 25 volunteers. Because sperm motility decreased by the second week, it was suggested that gossypol acts on the epididymal or testicular spermatozoa. Recovery of fertility for all 25 volunteers returned within three months of withdrawal. The reported side effects included decrease or increase in appetite, fatigue, dryness of mouth, diarrhea, tendency for sleepiness, and decreased libido. All these were reversible and generally mild. These side effects were reduced with a daily dose of 24-35 mg per day which was enough to maintain antifertility effects. The only concerning side effect was the reduction in serum potassium levels leading to hypokalemia in some of the subjects, which is why studies and clinical experiments were discontinued in the United States. However, the studies were continued up to a second and third trial in China. By 1980, they had over 8,000 volunteers. They found that the optimal dose to maintain antifertility was 20 mg/day for 60-70 days and 40-50 mg/week which corresponded to an antifertility efficacy of 99.07%. The hypokalemic paralysis side effect occurred with 0.75% of the subjects. Fertility returned to most volunteers after withdrawal of treatment; 10% took 6 months to 4 years to recover (Qian et al 1984).

From past studies, the ideal concentration of gossypol to induce interactions with the plasma membrane of spermatozoa was achieved with a single 20 mg dose that had a 10 day half-life. The lowest dose tried in clinical studies that was effective was a daily dose of 10 mg for three months. For these subjects, researchers observed that they had decreased sperm motility and density with no other side effects. In another study using 20 mg/day, the subjects were azoospermic two years after withdrawal of treatment (Porat 1990).



Gossypol interacts with glutathione peroxidase and glutathione reductase resulting in lipid peroxidation of spermatozoa.

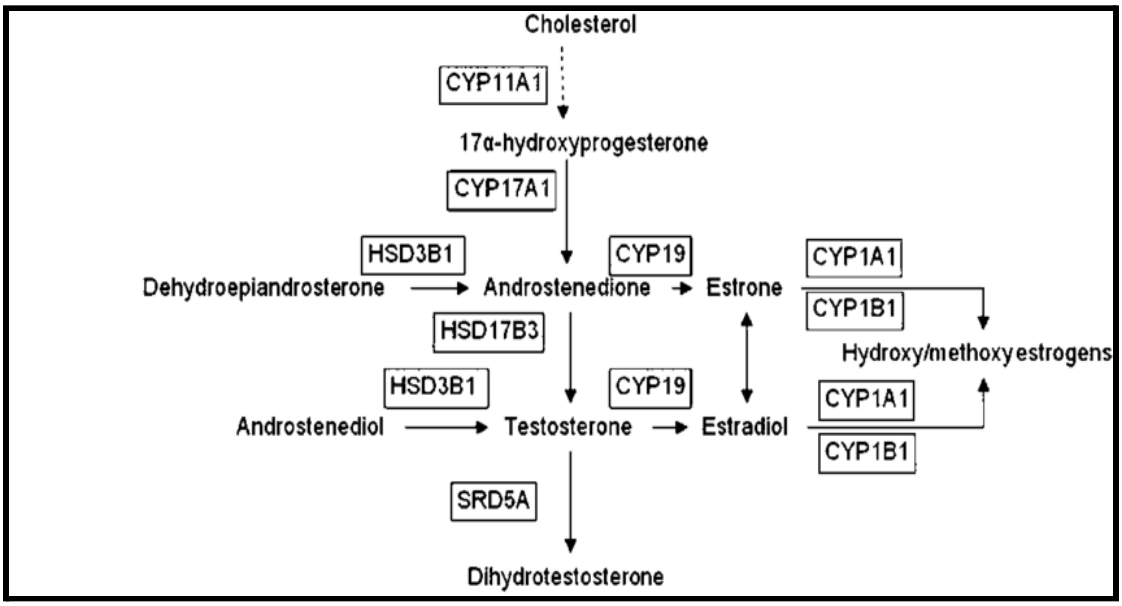
Literature has suggested that gossypol induces a decrease in sperm count by increasing the activity of glutathione peroxidase and glutathione reductase. The metabolic result was that there were reduced levels of glutathione and pyridine nucleotides in the testis along with increased levels of oxidized glutathione (Santana et al 2015). These effects were reversible with treatment of vitamin E which inhibited the action of gossypol. Based on these results as well as other primary literature, gossypol generates oxidative stress in spermatozoa by unbalancing the reactive oxygen species generated from aerobic metabolism and protective antioxidants. Reactive oxygen species influence the physiology of normal sperm function, acrosome reaction, and motility of spermatozoa. Spermatozoa are more vulnerable to reactive oxygen species compared to other cells as their plasma membrane and cytosol is rich in polyunsaturated fatty acids, and lipid peroxidation of the sperm membrane is known to cause sperm damage,and thus result in infertility (Adeoye et al 2018). As gossypol in the 1980s was known to result in reduced sperm motility and integrity, this biochemical mechanism is supported by experimental observation.

Gossypol, due to its toxic nature, most likely will not be applicable as a possible male contraceptive method in rural communities. However, because of its nearly 100% efficacy, recent studies have focused on developing methods to modify its metabolism in the body in order to prevent long-term consequences. Beyond the potential hypokalemia, in Chinese clinical studies, overall, gossypol was observed to be an efficient contraceptive and well-tolerated by most male subjects. However, gossypol when metabolized by the body over a longer period of time reduces the plasma potassium leading to hypokalemia. In addition, for about ten percent of the Chinese subjects, gossypol induced permanent infertility. To address these two issues, a male birth control pill with a zero-order release administration is proposed. The hope is that this administration could improve the efficiency by maintaining a constant plasma level such as female contraceptives like the NuvaRing, reduce the toxicity of the gossypol compound, and improve patient compliance and convenience. To create the zero-order release of gossypol, it was incorporated into the production of a layer-by-layer film. Polyethylene and gossypol were combined with a quartz slide (which served as a substrate) to create hydrogen-bonding between the two reagents. When the release kinetics of the film was tested, it demonstrated a constant release of gossypol which maintained a concentration of approximately 30 to 40 mg/mL over a twenty day period. With oral administration, in order to achieve antifertility effect, a daily dose of a minimum of 25 mg/kg needs to be administered to the male rats. However, with the 30-bilayer, despite only having approximately 3.74 mg of gossypol, it demonstrated a 70% decrease in motility of sperm and continuously released gossypol for twenty days performing more efficiently than oral administration. Based on this data, concerning the mice, a daily dose of 0.44 mg/kg, a 50-fold lower dose than most studies using gossypol, could be used to achieve infertility. In addition, with the mice treated with the 30-bilayer administration, their plasma potassium levels remained constant over the

twenty day period. Further, after the withdrawal of treatment, sex organ weight and sperm mobility return to baselevel after 12 weeks indicating that the mice regained their fertility (Wen et al 2017).

The Future of Phytoconstituent-Based Male Contraceptives

Azadirachtin and neem extract possess the strongest potential as a male contraceptive for rural communities and developing regions. As stated previously, the importance in the designing of a male contraceptive is that it is able to meet the needs of diverse communities. Thus, it becomes necessary to propose various non-hormonal methods.



Shown is the metabolic pathway for the production of testosterone. Treatment of Azadirachtin induces a reduction in testosterone. Thus, by investigating which enzyme in the metabolic pathway is inhibited by azadirachtin, additional studies can be done to determine the Michaelis constant and the human dosage of neem extract for male contraception.

Azadirachtin has already been safely proven to work as a potential bioactive molecule in a male birth control pill or as a single injection such as the female Depo shot counterpart. Both would address various needs of rural communities. However, in order to advance the research of neem extract, the human-dose equivalent must be ascertained. Determination of the human dose equivalent of compounds is performed by the Baur's mouse dose constant of 22.4 mg/kg multiplied by the Km of the mouse and divided by the Km of the compound for humans (Reagen-Shaw et al 2007). From past experiments with mice, effective dosages ranged anywhere from 50 to 500 mg/kg,

which by calculations suggests for humans a factor of 2-22 times more for humans. Because of the variant purity of azadirachtin in human consumption, there is no known value for effective doses in humans to achieve infertility. Based on Bansal et al research, they extrapolated that an effective concentration for humans would be a solution containing 10 to 25% of the bioactive azadirachtin (Bansal & Gupta 2010). From research, the Km for both mice/rats and humans is unknown. Km is an enzyme kinetic constant that describes the affinity of biological enzymes for a particular substrate. To determine the mouse enzymes of interest, enzymes involved in the production of testosterone and spermatogenesis should be biochemically screened and purified. There is no reported literature suggesting enzymes for which azadirachtin acts as substrate and produces an inhibitory effect. Thus, a biochemical assay must be performed by exposing the enzymes to azadirachtin to determine which interact with the substrate. Enzymes that interact with azadirachtin will then be isolated and the Michaelis-Menten kinetics of the enzyme and substrate will be measured experimentally. The Michaelis constant will be determined for each enzyme. This will be repeated for the same human enzymes. Then, the human-specific concentration for neem extract can be determined. The implication of this allows for clinical studies investigating the necessary dosage for human intraperitoneal and oral administrations of neem.

For the enzymes that are inhibited by azadirachtin, another biochemical assay will be performed to see if gossypol is able to inhibit that particular enzyme. If this assay is positive, another biochemical assay will be done using both azadirachtin and gossypol to confirm that the enzyme is inhibited by both substrates, thus ensuring that a contraceptive utilizing both compounds would be most efficient at inducing infertility.

Literature has suggested that gossypol acts as an inhibitor to lactate dehydrogenase-X, an isoenzyme in mature testicular epithelium and spermatozoa. It is responsible for the electrophoretic mobility property of spermatozoa. Thus, a biochemical assay needs to be performed to confirm this relationship. If the results are positive, then the Km should be determined. This data will suggest a pharmaceutical dosage of gossypol needed to inhibit spermatozoa mobility. The implications of this experiment is that azadirachtin, which inhibits testosterone production, can be integrated with gossypol, an inhibitor of spermatozoa motility, in a zero-order bilayer release design to create a non-hormonal male birth control pill. In terms of expected doses, based on the Wen et. al, they proposed that with a zero-order release bilayer, they could decrease the effective gossypol dose in rats by 6.68 times. In Qian et al Chinese human trials , 60-70 mg/kg proved effective at inducing infertility in males. However, simultaneously, this dose was associated with approximately 3% of the subjects developing side-effects. Thus, if the Wen et. al findings are applied to Qian et al, then only 8.98 to 10.48 mg/kg of gossypol would be needed to synthesize an effective zero-order release bilayer. This low of a dose would dramatically decrease potential gossypol-related side-effects.

To extrapolate these findings to a long-term male contraceptive method, the male version of the IUD can be designed. The most effective female birth control is the IUD. A long-term implant, it does not have user error. If the homologous medical device could be implanted in the vas deferens, it would be a convenient method of male contraceptive that could be long-term. In addition, theoretically, it could be administered once in a rural community by medical professionals as long as there was a follow up every so many years. Depending on the success of the zero-order release bilayer for gossypol, the device could be structured like the NuvaRing and release a controlled amount of bioactive molecules each day. The bilayer would allow for a minute amount of gossypol to be used, thus decreasing the potential for long-term consequence. As the potential side effects for vas deferens injections of azadirachtin are already known, the efficacy of the device could be enhanced by the addition of azadirachtin in the synthesis of the bilayer.

To integrate the findings of this project, a male birth control pill with a 10-25% mg concentration of azadirachtin either embedded or surrounded by a zero-order gossypol bilayer containing 8.98 to 10.48 mg/kg of active gossypol would prove an effective, relatively safe male contraceptive that would dual-inhibit spermatogenesis and sperm motility.

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