The Pursuit of Parenthood: Expanding Horizons of Reproductive Physiology and Assisted Reproductive Technologies

by

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ABSTRACT

The desire to start a family is something millions of people around the globe strive to achieve. However, many factors such as the societal changes in family planning due to increasing maternal age, use of birth control, and ever-changing lifestyles have increased the number of infertility cases seen in the United States each year. Infertility can manifest as a prolonged inability to conceive, or inability to carry a pregnancy full-term. Modern advancements in the field of reproductive medicine have begun to promote the use of Assisted Reproductive Technologies (ART) to circumvent reduced fertility in both men and women. Implementation of techniques such as In Vitro Fertilization, Intracytoplasmic Sperm Injection, and Pre-Implantation Genetic Testing have allowed many couples to conceive. There is continual effort being made towards developing more effective and personalized fertility treatments. This often begins in the form of animal research—a fundamental step in biomedical research.

This dissertation examines infertility as a medical condition through the characterization of normal reproductive anatomy and physiology in the introductory overview of reproduction. Specific pathologies of male and female-factor infertility are described, which necessitates the use of ARTs. The various forms of ARTs currently utilized in a clinical setting are addressed including history, preparations, and protocols for each technology. To promote continual advancement of the field, both animal studies and human trials provide fundamental stepping-stones towards the execution of new techniques and protocols. Examples of research conducted for the betterment of human reproductive medicine are explored, including an animal study conducted in mice exploring the role of tyramine in ovulation. With the development and implementation of

new technologies and protocols in the field, this also unearths ethical dilemmas that further complicate the addition of new technologies in the field. Combining an extensive review in assisted reproduction, research and clinical fieldwork, this study investigates the history and development of novel research conducted in reproductive medicine and explores the broader implications of new technologies in the field.

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ABBREVIATIONS

ACOG	American College of Obstetricians and Gynecologists
AI	Artificial Intelligence
AINSEM	Artificial Insemination
AIDS	Acquired Immunodeficiency Syndrome
AMH	Anti-Mullerian Hormone
APA	American Pregnancy Association
ART	Assisted Reproductive Technologies
BSA	Bovine Serum Albumin
CAP	College of American Pathologists
CAVD	Congenital Absence of the Vas Deferens
CDC	Centers for Disease Control and Prevention
CLIA	Clinical Laboratory Improvement Amendments
DAB	3,3'-Diaminobenzidine
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic Acid
EDO	Ejaculatory Duct Obstruction
ERICA	Embryo Ranking Intelligent Classification Algorithm
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
FSHR	Follicle Stimulating Hormone Receptor
GnRH	Gonadotropin-Releasing Hormone
hCG	Human Chorionic Gonadotropin

HH	Hypertrophic Hypogonadism
HHS	Health and Human Services (U.S. Department of)
HIV	Human Immunodeficiency Virus
hMG	Human Menopausal Gonadotropin
HPLC	High-Performance Liquid Chromatography
HSG	Hysterosalpingogram
ICM	Inner Cellular Mass
ICSI	Intracytoplasmic Sperm Injection
INSEM	Conventional Insemination
IP	Intraperitoneal
IUI	Intrauterine Insemination
IVF	In Vitro Fertilization
LH	Luteinizing Hormone
MAC	Monitored Anesthesia Care
NASS	National Assisted Reproductive Technology Surveillance System
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NICHD	National Institute of Child Health and Human Development
NHSR	National Health Statistics Report
NSDUH	National Survey on Drug Use and Health
NSV	No-Scalpel Vasectomy
OBGYN	Obstetrician Gynecologist
OCP	Oral Contraceptive Pill
OHSS	Ovarian Hyperstimulation Syndrome

OI	Ovulation Induction
OTC	Ovarian Tissue Cryopreservation
PBS	Phosphate-Buffered Saline
PCOS	Polycystic Ovarian Syndrome
PESA	Percutaneous Epididymal Sperm Aspiration
PMDS	Persistent Mullerian Duct Syndrome
PMSG	Pregnant Mare Serum Gonadotropin
POI	Primary Ovarian Insufficiency
PVP	Polyvinylpyrrolidone
RPL	Recurrent Pregnancy Loss
SART	Society for Assisted Reproductive Technology
SRY	Sex Determining Region
STI	Sexually Transmitted Infection
T4	Thyroxine
TAAR1	Trace Amine Associated Receptor 1
TDF	Testis Determining Factor
TESE	Testicular Sperm Extraction
THC	Delta-9-Tetrahydrocannabinol
TIC	Timed Intercourse
TSH	Thyroid Stimulating Hormone
TURED	Transurethral Resection of the Ejaculatory Duct
UTx	Uterine Transplant
WHO	World Health Organization

Reproduction is a complex and essential function that ensures the survival of a species over time. At its core, human reproduction involves the fusion of gametes—an oocyte from a female and a single sperm cell from a male. While the male and female reproductive systems function independently, they must ultimately work in tandem to achieve successful fertilization during sexual reproduction. Underlying the many anatomical complexities of the male and female reproductive system are hormones and other molecules that facilitate the processes of reproduction. Dysfunction of these mechanisms can lead to an inability to reproduce, clinically referred to as infertility.

Development of a more comprehensive understanding of the pathology of infertility has resulted in numerous improvements in human reproductive medicine—particularly through the establishment of assisted reproductive technologies [ARTs]. Initially, ARTs were created for use in the agricultural industry as a means for optimizing animal reproduction. Successful application of these protocols in animals led to the adaption of ARTs as a clinical approach to addressing human infertility. Since its inception, millions of babies have been born using ARTs across the globe. Still, there is continual effort being made towards improving upon these protocols and expanding treatment options available to patients. Foundational studies for the betterment of human reproductive medicine begin with laboratory research using animal models, with the eventual goal of implementing these practices in humans. There are four main aims of this dissertation: 1) *to describe how the reproductive system works to assess and treat disorders,* 2) *examine the development and use of fertility treatments and ARTs in reproductive medicine*, 3) *to analyze prominent ethical issues I observed in the*

field of reproductive medicine, and 4) *review novel methodologies that may advance the field of reproductive medicine*.

I implemented various methodologies to explore each of my aims: literature review, clinical fieldwork, and laboratory benchwork. To set up my dissertation, I conducted an extensive literature review to understand the climate of reproductive medicine within the United States [U.S.] and to establish a solid foundation of reproductive anatomy and physiology. This included review of landmark legislation that shaped reproductive rights for women in the U.S., as well as review of both anatomy and physiology literature to understand the biological processes related to reproduction. I used primary literature to identify original research published in the fields of reproductive biology, obstetrics and gynecology and urology, and utilized tertiary literature in the form of anatomy and physiology textbooks and dictionaries to characterize what constitutes "normal reproduction." This comprehensive review provides the foundation for Chapter 1: Assisted Reproduction: What is it, What are the Costs and What are the Issues, Chapter 2: Reproduction: An Overview, and Chapter 3: Diagnosis and Characterization of Reduced Fertility.

To better understand the inner workings of fertility medicine, I worked at a local fertility clinic for a year. The name of the clinic that I worked at will not be disclosed in this dissertation due to confidentiality and a non-disclosure agreement. I began my training as an andrologist where I learned numerous protocols and techniques including how to perform a semen analysis, to write diagnostic reports for male fertility testing and to conduct various methods for preparation and storage of semen for fertility treatments.

Andrology laboratory staff are also responsible for conducting blood serum immunoassays to analyze fertility hormones for both male and female patients.

Conducting these analyses further enhanced my understanding of reproductive physiology and the process of medicated ovulation induction that is often used during fertility treatment. My responsibilities as an andrologist at the fertility clinic also included assisting local urologists with outpatient procedures to address cases of male-factor infertility—particularly varicocelectomies and testicular biopsies. After mastering the protocols and procedures performed in the andrology lab, I was moved into the embryology lab at the clinic as an embryology trainee. Senior embryologists trained me on performing oocyte collection during patient retrievals, as well as techniques for oocyte handling, stripping, freezing, and thawing. I also gained exposure to micromanipulation of oocytes under the microscope for laser-assisted hatching and intracytoplasmic sperm injection. Under the direction of the embryology laboratory staff, I assisted with daily fertilization checks on embryos and began practicing embryo grading, biopsy, freezing and thawing—protocols which share many similarities with those used for oocytes.

The clinic performs between 60-80 patient cycles each month which expedited my training and allowed me to become proficient in many techniques within a few weeks. I draw upon my clinical experience in both andrology and embryology in Chapter 3: Diagnosis and Characterization of Reduced Fertility and in Chapter 4: Fertility Treatments and Assisted Reproductive Technologies. The clinical work I conducted was invaluable throughout my writing process and contributed to my ability to chronicle the history, development and techniques of different ARTs utilized in fertility medicine.

Specific preparations and protocols involved for each are described in detail, including the roles and responsibilities of patients, laboratory staff and clinicians, where applicable.

My experience as a laboratory staff member at the fertility clinic allowed me to garner substantial technical expertise in the field. In addition, I was also responsible for communicating results of a patient's fertility testing back to the patient (this is not to be confused with *interpreting* a patient's results, which is performed by the physician). Through my interactions with patients, I became aware of the current gaps in the overall understanding of human reproduction by patients seeking reproductive care. Chapters 1-4 are written for a general audience, particularly for people that are potentially interested in pursuing fertility treatment. These chapters are intended to outline the scope of diagnostic testing and treatments commonly performed at fertility clinics, characterize the pathology of infertility using medical terminology, and describe how different ARTs are carried out in the lab using written protocols and procedures.

As a member of the laboratory staff, I also noticed prominent ethical dilemmas present in the field of reproductive medicine that resurfaced often during my time at the clinic. Chapter 5: Ethical Dilemmas in Fertility explores several of these dilemmas, specifically regarding donation of sperm, compensation of oocyte donors, and the use and implications of pre-implantation genetic testing of embryos. This chapter combines a review of previous work with my unique perspective from a clinical standpoint. Understanding and addressing the current ethical challenges present in the field are instrumental in the process of developing and implementing new techniques in the field through scientific research.

Research for the betterment of human reproductive medicine is an ongoing effort. Scientific research can be conducted to improve upon an existing technique, develop a novel protocol, or to improve our understanding of the mechanisms of infertility. In Chapter 6: Current and Future Fertility Research, ongoing research in the field of reproductive medicine is explored and I examine the future directions of each study. This chapter covers research in several major areas of the female reproductive system, and a significant study conducted in the male reproductive system. These studies are at various stages of development and execution, but all have reached the stage of preliminary human trials. The process of developing novel therapies and treatment protocols for use in human medicine requires extensive testing prior to being approved for even preliminary human trials. Scientific research begins as pre-clinical animal or cell-line studies to establish safety and efficacy before receiving approval to advance to clinical trials in humans.

Even studies that do not reach implementation in humans still provide us valuable information and may even challenge what we believe know about human reproduction. The final method utilized in my research is laboratory benchwork in the form of animal research. Outside of the fertility clinic, I conducted an animal study at Arizona State University on the biogenic amine tyramine and its role in ovulation in mice, which is described in Chapter 7: Role of Tyramine in the Mouse Ovary. I utilized both qualitative and quantitative methods to compare the effects of physiological doses of tyramine on follicular maturation and ovulation including techniques such as High-Performance Liquid Chromatography, histology, confocal microscopy, and immunohistochemistry. Key findings and future directions of my study are described in this chapter, with

additional studies needed using human ovarian tissue to determine the potential role that tyramine may play in humans.

To conclude, the final chapter of my dissertation highlights the contributions that my unique set of knowledge and experiences provides to the field of reproductive medicine and the scientific field at large. While history, ethics, and scientific research all play important roles in the advancement of human medicine, my clinical experience was invaluable to the preparation of my dissertation. This work concludes with personal insight and interpretations into the future of fertility medicine as ARTs become a more prevalent method for conception.

CHAPTER 1

ASSISTED REPRODUCTION: WHAT IS IT, WHAT ARE THE COSTS, AND WHAT ARE THE ISSUES?

Synopsis

Chapter 1 provides an overview of the concept of reproduction—as a societal norm, an aspect of general health, and as a complex and essential biological function. First, I contextualize the sociological importance of reproduction and describe the evolution of reproductive rights in the United States over time. This provides the necessary framework for discussing the *need for* and *use of* fertility treatments as a means for promoting reproductive health in both men and women. After conducting a year of clinical fieldwork in fertility medicine, I became aware of the significant cost and limited coverage of these fertility treatments. I also noticed prices of receiving fertility services increasing, despite reported decline in success rates. The inverse relationship between cost and success was disconcerting to me and was not a topic discussed with patients at the clinic—which will be discussed in this chapter as well.

Reproduction and Society

Starting a family is something millions of people around the globe strive to achieve, which can be tied to personal, religious, cultural, and even family traditions. Over time, the definition of "family" has evolved to include far more than the traditional nuclear family. The term now encompasses single parent, blended, childless, grandparent, stepparent, and non-related families as well. While all these definitions of what constitute a family are valid, this dissertation will focus on building a family by means of having children through pregnancy or surrogacy. Millions of babies are born in the United States

each year with over 3.7 million babies born in 2018 alone (Martin et al., 2019). While this number seems substantial, the birthrate per 1000 women in the United States is the lowest it has been since 1986. Numerous factors have contributed to the decline in births over the last 35 years such as women waiting longer to get married, waiting longer to have children, or having smaller families altogether (Schmelz, 1976).

According to a study conducted by the Pew Research Center in 2018, there has been a significant shift in priorities for young adults over time (Fry et al., 2018). Beginning with the Silent Generation (individuals born between 1928-1945), only 15% of men and 9% of women ages 21-36 had completed at least bachelor's degree, compared to 22% of men and 20% of women in the Boomer Generation (born between 1946-1964) (Fry et al., 2018). The dramatic increase in educated individuals in the U.S. is further exemplified for Gen X. For the first time in U.S. history, more women between the ages of 21-36 had attained at least a bachelor's degree than men. This trend continues for Millennial women who are now four times as likely to have at least a bachelor's degree than women from the Silent Generation (Fry et al., 2018). The evolving role and perception of women in America from the traditional domestic role to a modern, career-focused one has also shaped the demographic landscape of the U.S. A combined analysis of Centers for Disease Control and Prevention [CDC] data and National Vital Statistics Reports conducted by Guzzo & Payne, reported that the average age of a woman's first childbirth in 1970 was 21.4 years, with a total fertility rate of approximately 2.48 births per woman. By comparison, in 2017 the average age of a woman's first childbirth was 26.8 years with a total fertility rate of 1.78 (Guzzo & Payne, 2018).

In addition to the pursuit of higher education as a factor impacting the birth rate in the U.S., normalization of oral and intrauterine contraceptive methods starting in the 1960's gave women more reproductive autonomy. Despite fervent disapproval from the Catholic Church, two landmark cases: *Griswold v. Connecticut* (1965) and *Eisenstadt v. Baird* (1972) established the right to contraception for married and unmarried couples. Several years later in 1972, *Carey v. Population Services International* (1977) granted the right of juveniles to have access to contraception as well. Being granted legal access to contraceptive methods did not diminish any of the social animosity experienced by the women who chose to utilize them, especially by members of secularized groups. Nevertheless, these cases were monumental in paving the way for women to have access to essential services needed to promote their overall health.

Health and Reproductive Medicine in the United States

The World Health Organization [WHO] defines health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (WHO, 1947). Health is regarded by the WHO as a fundamental human right, regardless of an individual's race, religion, political alignment, or socio-economic status. Incorporated into the definition of health is the right of both men and women to have equal access to safe, affordable, and effective fertility regulation, and the right for pregnant women to access appropriate reproductive health services throughout their pregnancy (WHO, 1947). Reproductive medicine is a growing field that encompasses all aspects of both male and female reproduction. This includes topics of physical well-being such as maternal and infant health, pregnancy, abortion, maternal mortality, contraception, and infertility as well as psychological well-being (CDC, 2019a). The

ultimate goal of the field of reproductive medicine is to improve sexual and reproductive health outcomes for both men and women, promote infant health, and educate patients on the ways in which they can optimize their health and understand their options for seeking care or treatment.

Following the groundbreaking cases regarding the legality of contraceptive methods, reproductive medicine was no longer limited to maternal-fetal medicine. Women could seek counsel regarding their reproductive health regardless of their childbearing status, openly discuss options for family planning with a healthcare provider and even delay starting a family using more reliable forms of medical contraception. Since 1970, worldwide use of contraceptives has nearly doubled—from just 35% of women in 1970 to 64% in 2015 (United Nations, 2015). Through their study, the United Nations demonstrated that the desire of women to have control over their own reproductive health is not unique to the U.S., or solely in developed countries. However, desire, access, and affordability of reproductive services are not equal everywhere. Differences can be found when comparing access to reproductive health services across geographical space—even in the U.S. where contraception is legal. A lack of access translates to a lack of reproductive rights. In 2015, the CDC reported that there were no significant differences in contraceptive use across varying education levels, and only an 8% variation across non-Hispanic white, non-Hispanic black, and Hispanic races. Still, sociodemographic characteristics largely contribute to whether an individual has access to these services at all, which is influenced by numerous factors including an individual's education, insurance status, and race/ethnicity (Krings et al., 2008). The Title X Family Planning Program was created in 1970 to help underprivileged women access

reproductive care (Kreitzer et al., 2021). Although administrative policies enacted since then have more often undermined the effectiveness of the program than improved it, leaving 19 million U.S. women in counties that are considered "contraceptive deserts." Contraceptive deserts are areas in which there is no reasonable access to a reproductive health center that offers a full range of contraceptive methods (Saunders et al., 2018).

Equal and affordable reproductive healthcare for women in the United States has been a long-fought battle—one that has yet to be resolved in its entirety. Ultimately the disparities seen in reproductive healthcare most significantly impact minorities and couples living at or below the poverty line (Saunders et al., 2018). Nonprofit organizations such as Planned Parenthood have stepped in to help bring access to these demographics but offer a more limited range of services compared to options provided at private clinics. Services provided by Planned Parenthood are centered around education regarding sexual health, providing birth control, emergency contraception and abortion services (Silver & Kapadia, 2017). However, there are not programs available to help women who are ready to start families but are struggling to conceive or maintain a pregnancy (Saunders et al., 2018).

Fertility Treatment Cost and Coverage in the United States

Infertility impacts roughly one in ten women of reproductive age in the U.S., but fertility treatment is seen as a luxury by insurers and very few states require any degree of coverage for fertility treatments at all, shown in Figure 1 (Devine et el., 2014). Initial consults, scans, and bloodwork that precede any medical intervention for fertility can cost hundreds of dollars out of pocket. At the clinic where I worked, baseline values are established for the patient first before fertility treatment can begin. This process that can

take up to a month to complete, depending on when the patient has their first appointment. Many diagnostic fertility tests must be completed on a specific day of the menstrual cycle, so patients are asked to return when their cycle starts over to begin testing. For women ready to conceive, Intrauterine Insemination [IUI] and In Vitro Fertilization [IVF] are the most common assisted reproductive technologies [ARTs] utilized in clinics. Hopeful couples experiencing less severe infertility or who are looking for a more cost-effective treatment may choose to pursue IUI's. IUI's are a minimally invasive procedure with an average cost of $3,000 depending on clinic location, use of artificial hormones, monitoring, and additional bloodwork performed (CNY Fertility, 2020a). In a report released by the American Pregnancy Association [APA], success rates for IUI's "may reach as high as 20% per cycle depending on variables such as female age, reason for infertility, use of fertility drugs, among other variables," although fertility clinics realistically report roughly 10% success for IUI's even when using fertility medications (APA, 2017).

Couples experiencing more severe infertility, or who have had repeated IUI failures may be recommended to pursue IVF—a more labor-intensive and invasive procedure with an even more substantial price tag. The average cost of a single IVF cycle in the U.S. is $20,000, which excludes the cost of any additional ARTs used such as intracytoplasmic sperm injection [ICSI], embryo biopsy for genetic screening, and yearly long-term storage fees for embryos (CNY Fertility, 2020a). Despite the considerable difference in cost, the CDC reports that on average the success after just one IVF cycle can range from 50-60%, which takes into consideration background, diagnosis, and obstetric history (CDC, 2019a).

For couples fortunate enough to not be omitted from receiving fertility treatment based on socioeconomic disparities, additional factors make reproductive medicine a difficult field to navigate for patients. Since fertility treatments are not typically covered by health insurance, patients are not limited to in-network providers or confined by state boundaries when searching for a physician to help them start their family. Research often begins with local fertility clinics using crowd-sourced reviews of each facility, physician and experiences with the supporting staff that are shared through online platforms. I have found that patients desire a personal relationship with their provider and do not want to feel as if the clinic is just an expensive revolving door.

The clinic that I worked with provided IUI and IVF cycles for costs that fall well below average. The clinic's IUI package included bloodwork, ultrasounds, injection teaching, and the IUI procedure itself for $750. A basic IVF package included bloodwork, ultrasounds, injection teaching, oocyte retrieval, assisted hatching, and all embryo transfer costs plus one year of embryo storage included for $5,000. A la carte options for IVF treatment included: intracytoplasmic sperm injection, embryo biopsy/genetic testing, and additional years of embryo storage. These cost-effective treatment plans offered by the clinic attracted patients from across the country, with many traveling to and from Arizona to receive treatment.

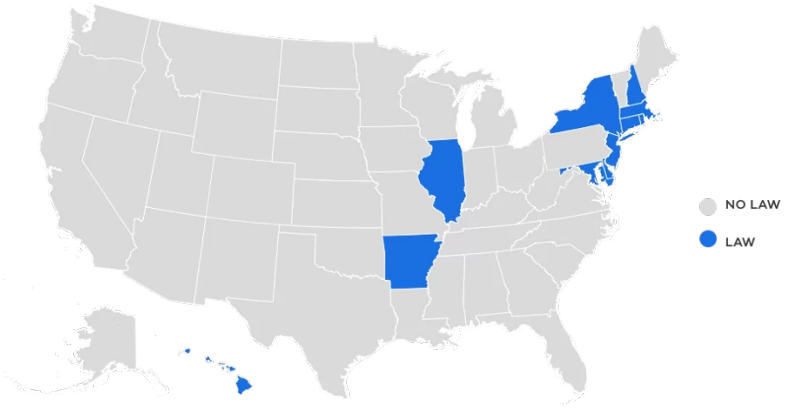

Figure 1. IVF Coverage by State. This map illustrates the specific states that have mandated some form of fertility coverage (CNY Fertility, 2020a).

Cycle Data Reporting: Defining "Success"

Even greater significance is placed on the "success rate" each clinic has—a value that complicates their search even further whether patients realize it or not. Without a concrete understanding of how success is defined by fertility clinics, patients can quickly be misled about how accurate a clinic's success rate truly is and misinterpret their chances of becoming the clinic's next testimony of success (Wiecki, 2018). So, what constitutes "success" and why are the definitions dissimilar between the clinics performing the work and the institutions reporting it? From the perspective of a fertility clinic, what is considered success of an IUI or IVF cycle is synonymous with pregnancy rate (Wiecki, 2018).

In the field of reproductive medicine, pregnancy rate is just one element of what constitutes an individual's obstetric history, commonly referred to as the GPA system. The GPA system is an acronym that stands for gravidity, parity, and abortion. Gravidity represents the number of times a woman has been pregnant; para, or parity refers to live

or still births that occur after 20 weeks of gestation; and abortion denotes fetal death in utero prior to 20 weeks of gestation (Creinin & Simhan, 2009). The GPA system holds merit in both the obstetric and fertility fields for diagnosing and treating reproductive disorders, but within the field of fertility specifically, an institution's role in the process influences which aspect of the GPA system holds the most value when determining what constitutes success (Creinin & Simhan, 2009). Fertility clinics use gravidity as their benchmark of success, while data reporting agencies see a live birth, or "para" as success, which more closely aligns with how patients would view success of their IUI or IVF cycle. Though it seems disconcerting that success is measured and reported differently in the same field, there is reasoning behind this approach. Variations in personal health and individual lifestyles from person to person can include factors known to impact pregnancy such as maternal diet, preexisting conditions, smoking, substance abuse, etc. (Sharma et al., 2013). Therefore, fertility clinics often use gravidity as their benchmark for clinical efficacy to reflect their expertise most accurately in using ARTs. This is because parity incorporates the reality that variations in lifestyle and obstetric history, largely out of a fertility clinic's control, could have been the reason for a pregnancy loss. Thus, in cases of pregnancies established through use of ARTs, it is assumed that pregnancy rate provides the most accurate and unabated reflection of the success of the laboratory staff at a clinic, as well as the success rate of the ART techniques themselves (Gleicher, 2018).

Establishing a pregnancy is a monumental milestone for patients struggling to conceive naturally and is indicative that the ART used has been successful. However, patients view confirmation of pregnancy as a mere steppingstone in the nine-month

waiting period to determine if their treatment was truly successful. When reporting annual fertility data, the CDC, and Society for Assisted Reproductive Technology [SART] regard success of IUIs and IVF cycles in the same way—conception is progress, but birth is a success. To distinguish between the definitions of success held by reporting agencies versus fertility clinics, the CDC and SART use "cumulative success," i.e. live birth rate, to describe success for ARTs (Kieu & Polyakov, 2021). Annual IVF data is compiled from all reporting U.S. fertility clinics, documenting age, race, diagnosis/reason for IVF and other relevant demographics. This information is used to generate an annual IVF success rate report—typically published two years after the reporting year (CDC, 2020).

Publishing these reports in arrears gives clinics ample time to follow up with patients to gather complete cycle outcome data (CDC, 2020). Data analysts from the CDC then generate tables for each clinic and for the U.S. overall. Information presented in fertility reports can include data such as: number of retrievals and transfers performed, live birth rate by age, fresh/frozen transfer data, and donor/non-donor information (CDC, 2020). There is some overlap between information published in CDC reports and SART reports, but SART's reports tend to focus more heavily on national birth data related to embryo transfer, while the CDC emphasizes patient volume for retrievals and transfers, and reason for using ARTs from clinic to clinic (CDC, 2021a).

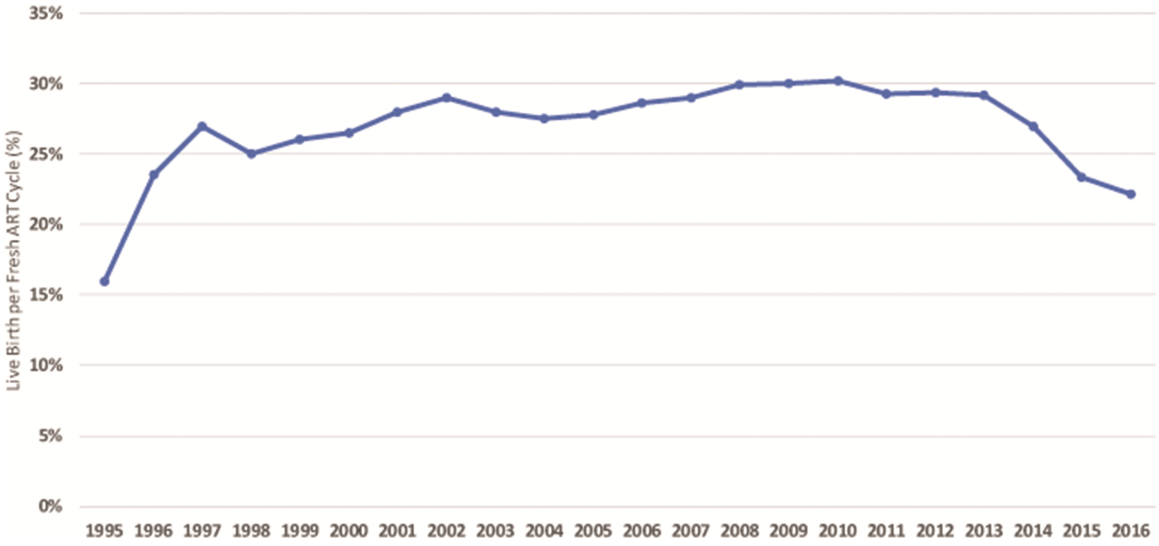

Figure 2. Live Birth Rates in the U.S.: Fresh Autologous IVF Cycles (1995-2016). (Gleicher et al., 2019).

Since the CDC began reporting this data in 1995, their number of fresh donor and nondonor retrieval cycles per year has nearly quadrupled (Gleicher et al., 2019). Using raw cycle data published by the CDC through the National ART Surveillance System [NASS], Gleicher, Kushnir, and Barad documented live birth rates from fresh autologous cycles from 1995-2016 (Figure 2). They noted: "Live birth rates demonstrate almost steady improvements until 2002, a decline between 2003 and 2007, reaching a new peak similar to that in 2002 between 2008 and 2010, only to again decline by 2016 to rates not seen since 1998" (Gleicher et al., 2019). This sudden decline in live birth rates from IVF cycles is largely unexplained. Based on the extensive reporting of cycle data in the United States from the CDC, the increased cost of IVF with lower success rates should be a cause of concern for patients interested in pursuing IVF.

Conclusion

Reproductive health is one of numerous aspects that is encompassed by the description of "health" according to the WHO. However, treating the various forms of reproductive dysfunction is often perceived as a luxury in the U.S. Despite coverage of fertility treatments being limited, more individuals are relying on assisted reproductive technologies such as IUI and IVF to expand their families. In this chapter, I established that reliance upon ARTs as a method for conception has evolved because of changing sociological factors that have led to women waiting longer to have children, as well as improved diagnostic and treatment capabilities in fertility clinics that allow physicians to address forms of reproductive dysfunction more accurately in both sexes.

CHAPTER 2

REPRODUCTION: AN OVERVIEW

Synopsis

Assisted reproduction is a complex topic. To understand and address the issues of infertility, the reader needs to have a good understanding of the biology involved. The diagnosis and treatment of reduced fertility requires a comprehensive understanding of reproductive anatomy and physiology—often called the "biology of reproduction." In this chapter, I describe the current understanding of the biology of reproduction beginning with early formation of the reproductive system during embryonic and fetal development. This will include post-pubertal reproductive anatomy and physiology to provide a foundation of understanding for how reproduction works under normal circumstances. Understanding what constitutes "normal" reproduction, will set the stage for discussing when reproduction does not go as planned—and reduced fertility becomes a reality requiring assisted technologies.

Early Embryogenesis and Development of Reproductive Anatomy

From the moment of fertilization where a single sperm and egg fuse to form a genetically unique zygote, fundamental processes of embryogenesis begin to occur. The initial mitotic divisions of the zygote catalyze a cascade of exponential cellular growth. The first division forms a 2-cell zygote, then 4-cell, and 8-cell within the first 72 hours following fertilization (Patrizio et al, 2003). When the zygote undergoes its fourth division resulting in 16 cells, it is then referred to as a *morula*, a Latin term meaning mulberry, given due to its resemblance to the small fruit (Hill, 2016). Division continues and the embryo enters the compaction stage which is characterized by the binding and

polarization of cells as they begin to differentiate and organize into distinct layers (Wolpert, 2007). The most superficial cells of the morula form what appears as an almost indistinguishable monolayer of cells called the *trophoblast* or trophectoderm, which will become part of the placenta. The remaining cells form a clump adhered to the trophectoderm called the inner cell mass [ICM] which will develop into the embryo (Patrizio et al, 2003). Following compaction of the trophectoderm and subsequent formation of the ICM, a fluid-filled cavity forms inside the embryo referred to as the blastocoele. The trophectoderm, ICM, and blastocoele are defining characteristics of the progression from the morula stage to the blastocyst stage (Figure 3). As the cells of the blastocyst continue to divide, the blastocyst becomes larger which puts increasing strain on the zona pellucida. As a result, the zona thins from the pressure of the growing blastocyst until a small hole forms in the zona, allowing the blastocyst to hatch out (Betts et al., 2013). Hatching is an important step and often facilitated manually in assisted reproduction as discussed later.

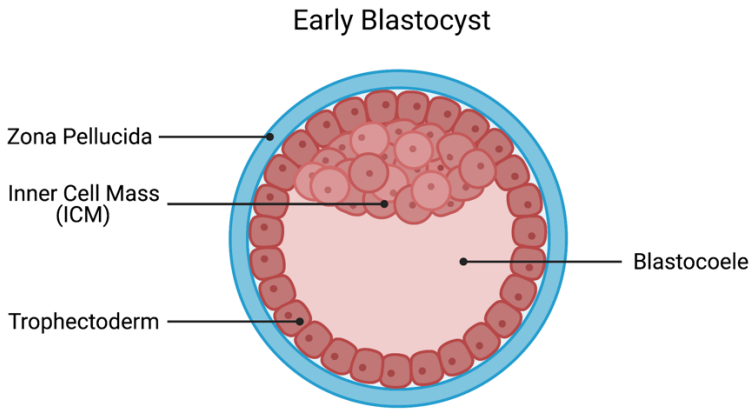

Figure 3. Early Blastocyst. This labeled diagram illustrates the different distinct cell types present in an early blastocyst. Created with BioRender.com

No longer under the space-constraints of the zona, the blastocyst can expand freely and is able to adhere to the uterine epithelium, then fully implant into the wall of the uterus to establish a pregnancy (Betts et al., 2013). Adhesion of the blastocyst to the lining of the uterus occurs between day six and seven post-fertilization (Betts et al., 2013). Small cilia present on the uterine lining roll the blastocyst over the surface of the epithelium until the ICM is closest to the lining. Once the ICM is orientated properly, complex endocrine signaling between the uterine epithelium and trophectoderm of the blastocyst initiate the implantation process, which is completed around day nine (Wilcox et al., 1999). Successful implantation of the embryo into the uterine wall between day seven and day nine post-fertilization is associated with a decrease in the likelihood of early pregnancy loss and initiates a surge in production of the hormone Human Chorionic Gonadotropin, or hCG (Wilcox et al., 1999). hCG plays a role in numerous developmental processes within the developing fetus such as: inciting angiogenesis, prompting maternal immunosuppression during invasion of trophectoderm cells into the uterine lining, blastocyst cell differentiation, and one of the most crucial roles in early development: promotion of placental growth (Cole, 2010). The placenta delivers vitamins, nutrients and water to the developing fetus and assists with maternal-fetal respiratory gas exchange, excretion, immune and endocrine functions necessary for healthy development, and is not fully formed until 14 weeks of embryonic development. (Kay et al., 2011).

During the third week of embryonic development, the first major differentiation of the ICM occurs called gastrulation (Bates & Bowling, 2012). Primarily associated with the formation of the gut, the process of gastrulation results in three layers of cells: the

ectoderm, endoderm, and mesoderm. Ectoderm cells contribute to the development of the nervous system, epidermis of the skin, adrenal gland, and both sensory and early endocrine structures (Hill, 2016). The endoderm is responsible for the development of both the gastrointestinal and respiratory tracts, auditory and urinary systems, as well as endocrine glands and organs such as the thyroid, liver, and pancreas (Gilbert, 2000). Between the endoderm and ectoderm lies the mesoderm. This layer forms a wide variety of connective tissues, the embryo's circulatory system, all three types of muscle tissue, the kidneys, and the reproductive system (Betts et al., 2013). While the mesoderm is formed during week three of development, further differentiation and specification of the human reproductive system does not begin until week nine (Hill, 2021). Up until this point, embryos possess an undifferentiated urogenital ridge, derived from intermediate mesoderm (Ortega et al., 2018). The urogenital ridge has two sets of ducts, one which would become the male reproductive system called the Wolffian duct, and the other would differentiate into the female reproductive system, the Mullerian duct (Ortega et al., 2018). This capacity of the urogenital ridge to become either the male or female reproductive systems is referred to as bipotentiality (Nef et al., 2019; Wilhelm et al., 2007). Further differentiation of this bipotential gonad into more definitive precursors for specific male or female reproductive structures relies on genetic sex-determination.

Like many other mammals, humans follow an XY sex-determination system. Humans have 46 individual chromosomes that contain all their genetic information—22 pairs of autosomal/non-sex chromosomes, and one pair of sex chromosomes (Betts et al., 2013). The sex of an embryo is usually determined upon conception—individuals with two X chromosomes are biologically female, while individuals with one X and one Y

chromosome are biologically male. The Y chromosome is smaller and contains fewer genes than the X chromosome; however the Y chromosome contains the single gene that controls sex determination—the sex-determining region Y [SRY] gene (Ortega et al., 2018). The SRY gene is responsible for the initiation of the production of testis-determining factor [TDF]. TDF promotes differentiation of the primitive sex chords formed by the urogenital ridge to become the testis, Wolffian/mesonephric duct, and other structures associated with the male internal genital tract (Jin et al., 2016). In the case of an embryo that is female with two X chromosomes and no Y chromosome, since the SRY gene is not present, TDF is not produced and the urogenital ridge differentiates into the Mullerian/paramesonephric ducts, ovaries, and other associated female reproductive structures (Wilhelm et al., 2007). Therefore, biological sex determination in humans is dependent upon the presence of a Y chromosome and subsequent activation of the SRY gene (Ortega et al., 2018).

Having two sex chromosomes is considered normal in humans, but in rare cases, abnormal sex chromosome combinations may occur. Turner Syndrome, also called Monosomy X is a condition characterized by one X sex chromosome and an incomplete or completely missing second sex chromosome, the child is born with female internal and external genitalia (U.S. National Library of Medicine, 2020). Turner syndrome leads to abnormal gonadal development which often results in severe infertility, other physical abnormalities such as stunted growth, skeletal and cardiac defects, swelling of extremities at birth, and potential learning disabilities (U.S. National Library of Medicine, 2020). Abnormal chromosome combinations can also include individuals with three or more sex chromosomes, such as: Klinefelter Syndrome where an individual can have sex

chromosomes XXY, XXYY, or XXXY; Supernumerary Y Syndrome/XYY Syndrome; Triple X Syndrome; or Pentasomy X Syndrome, a severe condition characterized by having five X sex chromosomes. Genetic disorders such as these and cases of hermaphrodites in humans do impact an individual's reproductive health and often their ability to conceive as well (Goncalves et al., 2017). These uncommon circumstances further complexify reproductive medicine and are important to acknowledge and take into consideration when developing care plans for patients seeking fertility treatment. However, discussing the individual genetic idiosyncrasies of underlying chromosomal abnormalities is beyond the scope of this dissertation. Going forward, the term *male* will be used to describe an individual with XY sex chromosomes possessing typical male reproductive anatomy comprised of only male reproductive organs, while the term *female* will describe an individual with two X sex chromosomes displaying typical female reproductive anatomy with solely female reproductive organs.

Human Reproductive Anatomy & Physiology

Development of the male and female reproductive systems continues in the fetus throughout pregnancy and is not complete until the third trimester (Betts et al., 2013). The sex of an embryo cannot be determined via ultrasound until 14 weeks of gestation at the earliest, but it is common practice to wait until 18-20 weeks' gestation to predict the sex more confidently (Odeh et al., 2009). Even after the internal and external reproductive structures have fully formed, the human body is not capable of sexual reproduction until the completion of puberty. Puberty occurs during adolescence in humans and is defined as "the morphological and physiological changes that occur in the growing boy or girl as the gonads change from the infantile to the adult state" (Marshall

& Tanner, 1986). Females typically enter and complete puberty earlier than males, but the onset of puberty is an individualized process that is influenced by both intrinsic and extrinsic factors, so timing and duration of puberty is highly variable (Sørensen et al., 2012). Changes associated with entrance into the pubertal stage are induced by natural production and conversion of sex hormones in the body by the adrenal glands and sex-specific gonads (Betts et al., 2013). Production of sex hormones promotes development of secondary sex characteristics such as pubic and facial hair, widening of hips and breast development in women, as well as enlargement of the Adam's apple in men (Hill, 2019).

Once an individual reaches the puberty stage, the hypothalamus in the brain increases production of gonadotropin-releasing hormone [GnRH]. GnRH acts on the pituitary gland to begin the release of luteinizing hormone [LH] and follicle-stimulating hormone [FSH] (Hill, 2019). Depending on the sex of the individual, LH and FSH will either trigger production of testosterone in the testes or estrogen and progesterone in the ovaries (Betts et al., 2013). Testosterone is the primary sex hormone for males and in addition to its role in the initiation of puberty, testosterone also plays a continual role in spermatogenesis for men throughout their lifetime. The average age of the first ejaculate containing mature sperm in males is around 13 years of age, which falls within the age range that boys experience puberty (Laron et al., 1979; Sørensen et al., 2012). For females, estrogens and progestogens are the fundamental sex hormones initiating changes during puberty—both of which are primarily produced in the ovaries. Normal menstruation in females follows a cyclic monthly pattern controlled by fluctuating levels of sex hormones and sex steroids. The first menarche in females is indicative that sufficient levels of estrogen are being produced in the body to initiate the growth and

shedding of the uterine lining, which typically begins between ages 12 and 13 (Likis & Schuiling, 2016). Variations of androgens, estrogens, and progestogens can be found in both sexes at all times; however, the functions and quantities of these sex hormones change depending on sex, age, and stage of the menstrual cycle if applicable. Reproductive hormones and other supporting molecules within the male and female reproductive systems will be described in further detail in Tables 1 and 2 in the following subheadings.

Male Reproductive Anatomy & Physiology

Following activation of SRY, primitive testes begin to develop from the Wolffian ducts (Wilhelm et al., 2007). Production of testosterone and anti-Mullerian hormone within the testes leads to degradation of the Mullerian duct, which otherwise would have become female reproductive structures (Betts et al., 2013). Once the Mullerian duct begins to degrade, the internal and external male reproductive organs begin to develop (Ortega et al., 2018). The penis, scrotum, testes, and epididymis are the major external male reproductive organs, with the vas deferens and other accessory organs housed internally (Figure 4).

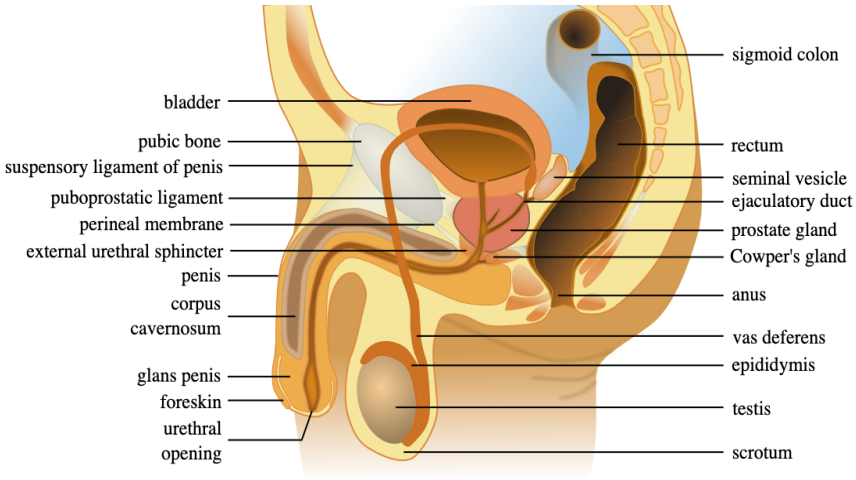

Figure 4. Male Anatomy. From Male Anatomy [Photograph], by Tsaitgaist, 2009, Wikimedia Commons (https://commons.wikimedia.org/wiki/File:Male_anatomy_en.svg). CC BY-SA 3.0

Each reproductive structure serves a unique role in the process of reproduction, but the principal function of the male reproductive system is to produce mature sperm that are capable of fertilization. Spermatozoa, or mature sperm, are the smallest human cell type and have a unique shape and chemical composition that aides in their ability to move and burrow into the zona of the oocyte (Millan et al., 2012). After formation of the testes is complete at 22 weeks of gestation, the testes descend from the pelvic cavity down to the scrotum—a process that requires the remainder of the gestational period to complete. Successful descent of the testes to the scrotal sack is an important milestone in male development (Betts et al., 2013). The scrotum is a skin-covered muscular sack that helps keep the testes at an optimal temperature for sperm development. The typical human body temperature is roughly 98 to 99°F, while the optimal temperature for sperm production is several degrees lower at 95°F (Fox & Van De Graff, 1992). Variations in

environmental temperature cause the scrotum to relax or contract—relaxing to move the testes further from the body if it is too warm or contracting and bringing the testes closer to the body when cold (Fox & Van De Graff, 1992).

Spermatogenesis, or the production of sperm within the testes is not initiated until the onset of puberty. Prior to the pubertal stage, the testes contain only diploid progenitors for sperm called spermatogonium, which contain the same number of cells as body cells—46 chromosomes (Betts et al., 2013). These precursors of sperm production lie dormant until LH and FSH levels rise, indicating that these hormones are actively being released by the pituitary gland—one of the major hormonal changes in puberty (De Kretser et al., 1998). Production of sperm takes place in the seminiferous tubules of the testes, with the initial mitotic division of the spermatogonium to form primary spermatocytes—these cells are also diploid and only half continue to divide, which ensures that there are always diploid cells serving as a reserve for spermatogenesis (Betts et al., 2013). Primary spermatocytes not serving as a reproductive reserve undergo meiosis to form haploid secondary spermatocytes. The term haploid means that the cell contains half of the number of chromosomes that a body cell has. A second round of meiosis occurs; meiosis II, yielding haploid spermatids. Spermatids will not undergo any further divisions but are still considered immature and incapable of fertilization. To become capable of fertilization, spermatids must undergo the process of maturation spermiogenesis (Betts et al., 2013). Spermiogenesis occurs in the epididymal ducts that connect to the seminiferous tubules and is characterized by the development of sperm polarity and subsequent transformation of spermatids into mature spermatozoa (Nishimura & L'Hernault, 2017). Spermatozoa possess a characteristic oval-shaped head

with a distinct acrosomal cap and flagellum tail (Nishimura & L'Hernault, 2017). Spermatozoa are stored in the epididymis in preparation to be ejaculated as a component of semen. However, if no ejaculation occurs within 24-36 hours, the spermatozoa will be naturally broken down by the body. A single complete cycle of spermatogenesis takes approximately 64 days with a new cycle beginning every 16 days (Betts et al., 2013). The cycle of sperm production begins during puberty and continues throughout the entire male lifespan.

In the event of sexual arousal, the penis becomes erect, triggered by the parasympathetic division of the autonomic nervous system (Alwaal et al., 2015; Hsu & Liu, 2018). The autonomic nervous system regulates numerous bodily functions, but erections are primary influenced by increased cardiac and vasomotor activity (Hsu & Liu, 2018). Together, increased heart rate and dilation of the arteries in the penis intensify blood flow, causing the penis to become erect and initiate muscle spasms. These contractions radiate through the reproductive tract back to the epididymis, pushing the stored spermatozoa up into the vas deferens, or ductus deferens (Alwaal et al., 2015). The ampullar region of the vas deferens is lined with secretory seminal vesicles that produce seminal fluid, the primary component of semen by volume that is rich with fructose, a source of energy for motile sperm (Betts et al., 2013). After lubrication from the seminal vesicles, further contractions move the semen through the ejaculatory duct. Enzyme-rich secretions from the prostate gland mix with the semen and increase its pH to help neutralize the acidity of the vagina (Barrett et al., 2019). The semen then travels through the urethra in the penis to be ejaculated (Hsu & Liu, 2018).

The penis is the prominent sexual organ in males that is composed of highly vascularized and innervated tissue that serves two primary functions: expulsion of semen and excretion of urine (Hsu & Liu, 2018). Both urine and semen pass through the urethra to exit the body. Urethral sphincters controlled by the autonomic nervous system prevent semen and urine from mixing during ejaculation—an involuntary yet crucial protective measure performed to protect sperm (Alwaal et al., 2015). Urine is a naturally acidic liquid waste product generated by the kidneys with a typical pH of around 6.2 (Rose et al., 2015). With a pH of around 6.0, urine is considerably more acidic than semen which typically has a pH of between 7.2-8.0 (WHO, 2010). If the urethra contains any residual urine when the ejaculate passes through, this can lead to a drop in semen pH and subsequent decrease in the viability of the sperm. Secretions from the prostate and bulbourethral glands add alkalinity to semen, protecting the sperm if any residual urine is left in the urethra prior to ejaculation (Alwaal et al., 2015).

Ultimately, regulation of the male reproductive system begins at the molecular level. Underlying all the anatomical complexities of the male reproductive system are hormones and other molecules that are working in unison to facilitate or suppress spermatogenesis, degradation of sperm, maintenance of secondary sex characteristics, and erections. These molecules, their site of production within the human body and their unique roles within the male reproductive system are outlined in Table 1.

Female Reproductive Anatomy & Physiology

In contrast to the development of the male reproductive system, embryos with two X chromosomes develop female reproductive structures derived from the Mullerian duct. Due to the absence of the SRY gene, degradation of the Wolffian duct follows (Ortega et

al., 2018). Most structures in the female reproductive system are housed within the pelvic cavity and do not move during development or puberty. Situated externally is the vulva, a collective term that encompasses several structures which serve protective, stimulatory, and lubricative functions that are considered accessory to the major internal structures (Betts et al., 2013).

The most external structures of the vulva are the labia majora and minora—two sets of lips that sit on either side of the vaginal opening. Together, the two sets of lips protect the urethra and entrance into the female reproductive tract (Betts et al., 2013). Also included as part of the vulva is the clitoris, a highly innervated organ covered by a thin fold of skin called the prepuce (Puppo, 2012). The clitoris is the primary source of sexual sensation for women and is derived from the same cells that would have formed the tip of the penis in males (Puppo, 2012). Anterior to the clitoris sits the urethral opening flanked by the Skene's glands. As with the male urethra, the female urethra serves the same excretory function, however the female the urethra connects solely to the urinary bladder, separate from the vagina (Betts et al., 2013). Skene's glands are small secretory glands that produce a clear lubricant which keeps the urethra from contracting any infections (Berkeley Wellness, 2013). Directly below the urethra and skene's glands is the vaginal opening and adjacent Bartholin's glands. The vagina serves as both the entrance and exit of the female reproductive tract. It is capable of expansion and contraction due its structure comprised of columns of muscular tissue—its unique composition is essential to accommodate intercourse and childbirth (Betts et al., 2013). During sexual arousal, the vaginal opening is lubricated by secretions of mucus by the Bartholin's glands, homologous to the bulbourethral gland in men (Lee et al., 2014).

Moving into the vaginal canal, the pH drops to an acidic 4.5 due to the presence of lactic acid (Betts et al., 2013). The vagina is a host for a plethora of beneficial bacterial flora, but is dominated by the presence of *Lactobacillus* bacteria, which secrete lactic acid as a byproduct of the metabolism of glucose. *Lactobacillus* bacteria play an important role in the self-cleansing properties of the vagina by preventing colonization of bacterial pathogens (Gong et al., 2014). At the top of the vaginal canal sits the cervical canal, which serves as the entry point of sperm into the uterus (see Figure 5). The cervix produces mucus that changes in consistency during different times of the menstrual cycle. During the ovulatory phase, cervical mucus becomes thinner due to a higher concentration of estrogen in the body (Ludmir & Sehdev, 2000). This facilitates movement of sperm in the semen from the vaginal canal, through the cervix and into the uterus (Betts et al., 2013). The body of the uterus is comprised of three layers of smooth muscle fibers that possess remarkable elasticity that can grow with and nourish a developing fetus. At the top of the uterine body, two fallopian tubes join the uterus on each side. These tubes are the passageway for oocytes, the female gamete, to pass from the ovary to the uterine body (Betts et al., 2013). Fertilization of the oocyte occurs in the portion of the fallopian tube closest to the uterus, as an unfertilized oocyte lasts a very short period of time after being ovulated (Bates & Bowling, 2012). The process of fertilization and "normal" reproduction will be described in more detail in the next subheading.

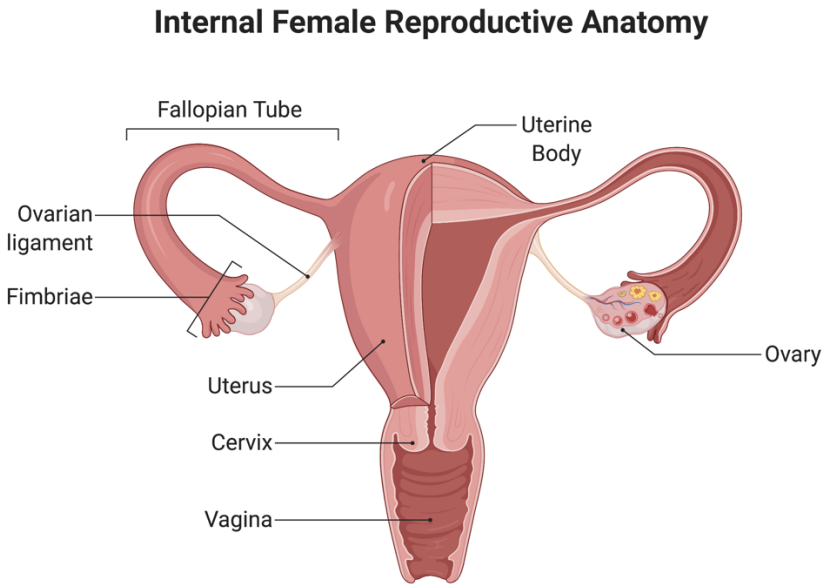

Figure 5. Internal Female Reproductive Anatomy. This labeled diagram shows the various internal structures of the female reproductive system. Created with BioRender.com

At the proximal end of the fallopian tube are numerous finger-like projections that extend towards the ovary called fimbriae (Bates & Bowling, 2012). These projections sweep the ovulated oocyte into the fallopian tube, where contractions in the smooth muscle in the fallopian tube and beating of cilia that line the tube move the oocyte away from the ovary towards the uterus (Bates & Bowling, 2012). The ovaries are complex, vascularized organs that are fully formed in the embryo by 22 weeks of gestation. There are two dynamic sets of processes that occur within the ovary: oogenesis and folliculogenesis. Oogenesis refers to the production of female gametes, oocytes, while folliculogenesis is a related process involving the development of ovarian follicles (Betts et al., 2013). As with sperm, oocytes are haploid cells that contain only 23

chromosomes—only after penetration by a spermatozoon will the genetic information contained in the newly formed zygote be complete (Patrizio et al., 2003).

During fetal development, progenitors of oocytes called oogonia form. Oogonia, like spermatogonium in the testes are diploid cells that first undergo a round of mitosis (Bates & Bowling, 2012). This initial mitotic division of oogonia yields diploid primary oocytes that begin the process of meiosis but are arrested in prophase and do not complete meiosis (Gilbert, 2000). Primary oocytes in the fetal ovary remain arrested in prophase of meiosis I until after puberty and are finite in number (Bates & Bowling, 2012). Years later following the onset of puberty, cyclic increases of LH within the ovary corresponding with the ovulatory phase of the menstrual cycle will initiate resumption of meiosis in a handful of primary oocytes at a time (Betts et al., 2013). The primary oocyte continues through meiosis from prophase and divides to form a single secondary oocyte and one first polar body. At this point, this secondary oocyte is now haploid with 23 chromosomes and arrests in metaphase II until fertilization following ovulation from the ovary (Patrizio et al, 2003). The accompanying first polar body may complete meiosis on its own to form two second polar bodies, but regardless of any further division, polar bodies generated through meiosis will eventually disintegrate (Betts et al., 2013).

Development of each ovarian follicle is closely linked to the process of oogenesis. From birth, each individual primary oocyte is surrounded by a flat and sparse monolayer of granulosa cells that will lie dormant until puberty (Bates & Bowling, 2012). These inactive follicles are referred to as primordial follicles and can remain inactive until menopause if they do not receive any hormonal signaling to begin maturation (Betts et al., 2013). Primordial follicles are the dominant follicle type found in the human ovary

throughout life, as only a handful of follicles mature at a time (Bates & Bowling, 2012). Following activation, the primordial follicle begins the process of maturation which lasts around 14 days (Betts et al, 2013). The majority of follicles that begin maturation will not end with the rupture of the follicle resulting in ovulation—rather, the cycle often ends prematurely due to follicular atresia where most follicles break down and one dominant follicle survives to the point of ovulation (Bates & Bowling, 2012; Betts et al., 2013). Follicular atresia is a mechanism of reproductive regulation, limiting the likelihood of multiple gestation by preventing multiple oocytes being ovulated at once (Hsueh et al., 1994). Breakdown of the follicle can occur at any time during folliculogenesis, and the remnants of the follicle and oocyte are reabsorbed.

Transition of the inactive primordial follicle to an active primary follicle involves several morphological changes that are influenced by complex signaling pathways of hormones and growth factors (Bates & Bowling, 2012). First, the thin, flat monolayer of granulosa cells begins to grow in size and rearrange into a denser formation of one or two layers of tightly packed cuboidal cells (Betts et al., 2013). The immature oocyte within the follicle starts to secrete a glycoprotein polymer that will eventually form the zona pellucida that surrounds the oocyte (Wolgemuth et al., 1984). Advancement into the secondary follicle stage involves intricate signaling within the oocyte that initiates recruitment of theca cells to the outermost layer of the oocyte and vascularization of the follicle. Theca cells are endocrine cells that produce precursors necessary for the synthesis of androgens and provides structural integrity, while vascularization forms a capillary net which transports nutrient-rich blood to and from the developing follicle (Young & McNeilly, 2010). The zona pellucida that began to take shape during the

primary follicle stage grows in thickness and is fully formed by the end of the secondary stage (Patrizio et al, 2003).

The final stage of follicular maturation has been given several names: the tertiary, antral, or Graafian follicle. The last milestone folliculogenesis is characterized by the presence of a large fluid-filled cavity adjacent to the oocyte called the antrum (Betts et al., 2013). Tertiary follicles contain no new cell types, and the oocyte is arrested in metaphase II awaiting ovulation. The end of the follicular stage is marked by a surge in production of LH by the anterior pituitary gland that triggers ovulation (Bates & Bowling, 2012). During ovulation, the follicular border closest to the surface of the ovary ruptures and the oocyte is expelled (Bates & Bowling, 2012). Left behind are theca cells that luteinize to form a small clump of progesterone-secreting cells called the corpus luteum during the luteal phase which lasts roughly two weeks. Higher levels of progesterone serve as an indicator for the uterus to prepare for implantation of a zygote—if no implantation occurs, progesterone levels decline as the corpus luteum degrades and the uterus sheds its lining as a part of menstruation.

Menstruation and the later stages of folliculogenesis are reliant upon routine cycling of FSH, LH, estrogen, and progesterone (Bates & Bowling, 2012). There are three stages of the menstrual cycle: the menses, proliferative and secretory stages (Betts et al., 2013). The menses and proliferative stages correspond with the follicular phase of the ovulation cycle. Shedding of the uterine lining or *endometrium* during the menses phase lasts two to seven days, followed by rebuilding of a new uterine lining during the proliferative phase lasting an additional week. The secretory phase of the menstrual cycle is the last and longest phase. As a response to the surge in progesterone levels produced

by the corpus luteum in the ovary, the endometrial lining of the uterus prepares for implantation of a fertilized oocyte. Lining the endometrium are tubular glands that secrete glycogen during the secretory phase. Glycogen serves as a glucose reservoir for nourishing an implanting zygote and is crucial during early embryonic development (Dean, 2019; Betts et al., 2013). If no implantation occurs, the corpus luteum degrades into ovarian scar tissue, the corpus albicans, until it can be broken down fully. Progesterone and estrogen levels decline and the lining sheds, starting the menstrual cycle over at the menses stage. Functional roles of the various hormones and molecules that regulate oogenesis, folliculogenesis and menstruation within the female reproductive system are outlined in Table 2.

Fertility and "Normal" Conception

For natural conception to be possible without use of ART or injectable medications influencing the likelihood of conception, both the male and female partners must be fertile. One of the most common misconceptions in reproductive medicine is that a patient can only be fertile or infertile, when in actuality there are four clinical descriptors of reproductive health: fertile, sub-fertile, infertile, and sterile. The latter three terms will be discussed in greater detail in the following subheading. However, it is easier to understand what makes a patient infertile by characterizing what constitutes "normal" fertility and conception beforehand. Fertility is the natural capacity for an individual to reproduce. Even under the best of circumstances, conception requires precise endocrinological timing to be feasible, made apparent by the complexities of spermatogenesis, oogenesis, folliculogenesis and menstruation discussed in this chapter.

Within the average 28-day span of the menstrual cycle, there are roughly five to six days in which a woman can become pregnant—although this value can vary slightly depending on how long a woman's menstrual cycle lasts (Wilcox, 2000). Therefore, women are fertile for approximately 60 days per year, while men do not experience the same cyclic regulation of their fertility. The capacity of an individual to reproduce also declines with age for both men and women, however this "fertility drop-off" is far less severe for men than it is for women. Men can produce mature sperm until the end of life, assuming no unforeseen disorders or diseases that would adversely affect their fertility. Women however undergo a total cessation of the menstrual cycle resulting in complete infertility, *menopause*. Most women enter the menopausal phase between 49 and 52 years of age (Takahashi & Johnson, 2015). Given this timeframe, between the onset of puberty and completion of menopause there is a roughly 40-year span that constitutes the period of a woman's "childbearing years." During this time when a woman is experiencing regular menstrual cycles, the four days leading up to ovulation and the day of ovulation itself are referred to as the fertile window (see figure 6)—the time in which a woman is most likely to conceive (Wilcox, 2000).

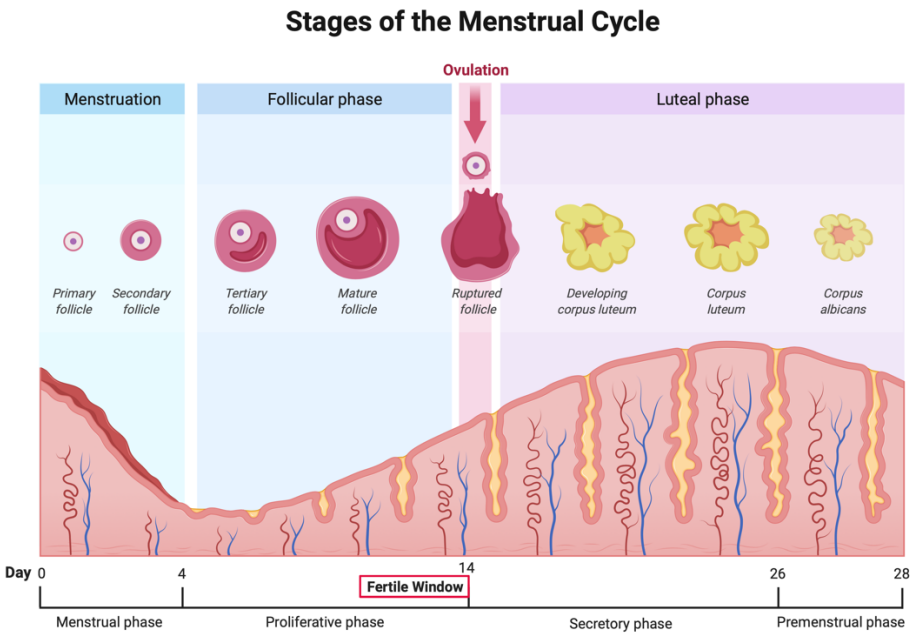

Figure 6. Stages of the Menstrual Cycle. The 5-day "Fertile Window" is marked in red on the timeline between, corresponding to days 9-14. Created with BioRender.com

Conception during the fertile window begins with sexual intercourse and ejaculation of semen into the vagina. The small motile sperm have a long journey to reach the ovulated oocyte—through the cervical canal, into the uterine body and up to the distal portion of the fallopian tube where fertilization occurs (Bates & Bowling, 2012). Many of the sperm cells in the ejaculate are killed off prematurely by the acidity of the vagina or are not moving swiftly or unidirectionally to be able to pass through the cervical mucus (Betts et al., 2013). The more limited number of sperm that do make it through the cervix undergo priming, or *capacitation*—an important process that involves thinning of the sperm's membrane to promote the acrosomal reaction between the sperm and oocyte during fertilization (Patrizio et al, 2003). Capacitation, combined with uterine

contractions facilitate movement of the sperm towards the fallopian tubes which can take 30 minutes to several hours (Suarez & Pacey, 2005; Betts et al., 2013). Sperm can survive in the fallopian tube for several days awaiting ovulation of an oocyte, but unused sperm will ultimately be phagocytosed in the distal portion of the fallopian tube (Suarez & Pacey, 2005).

After ovulation of a mature oocyte, the oocyte is swept into the fallopian tube towards to uterine body. Once inside the fallopian tube, cilia that line the fallopian tube roll the oocyte towards the uterus (Bates & Bowling, 2012). Sperm that have made their way to the distal portion of the fallopian tube rush towards the oocyte and burrow through the follicular cells still adhered to the outside of the oocyte. The first sperm to burrow through and begin penetration of the zona pellucida of the oocyte triggers the acrosomal reaction, releasing a digestive enzyme that dissolves the zona below it (Patrizio et al., 2003). Fusing of the sperm and oocyte triggers a chemical change in the composition of the zona, making it hard and impenetrable by any other sperm to prevent polyspermy (Patrizio et al, 2003). The fertilized oocyte is then referred to as a zygote and can complete meiosis from its arrested point in metaphase II (Betts et al., 2013). A woman's capacity to conceive and carry a pregnancy to full-term are controlled by numerous dynamic processes. Complications with either may lead to a diagnosis of reduced fertility.

Subfertility, Infertility & Sterility

With a foundational understanding of how the human reproductive system works, we can transition to sub-fertility, infertility and sterility which are clinically referred to as reduced fertility. Any extended period of time without successful conception or

maintenance of a pregnancy would be regarded as reduced fertility. Until fertility testing is conducted for both partners, the extent of reduced fertility cannot be explicitly defined, as there are distinct clinical differences between each condition. For *subfertile* patients, natural conception is still a possibility, but it is taking longer than expected to conceive (Gnoth et al., 2005). Mistakenly used interchangeably with subfertility, *infertility* is a more severe form of reduced fertility, defined as the inability to conceive after a year or longer of unprotected sexual intercourse, where medical intervention will be necessary to conceive (Chandra et al., 2013). In a 2013 National Health Statistics Report [NHSR] on impaired fecundity in the U.S. from 1982-2010, over 12% of women between the ages of 15-44 have experienced impaired fecundity, and this percentage increases with age. The NHSR also reported that in the United States alone, over 7.3 million women experience consistent complications with conception and are considered infertile. However, infertility is not solely a "woman's issue"—approximately half of all recorded infertility cases are due to male-factor infertility, and the other half are related to female-factor infertility (Kumar & Singh, 2015). Subfertility and infertility are quite similar in that both conditions can be caused by varying severity of some of the same reproductive disorders and dysfunctions. Sources of reduced fertility can include anatomical, endocrine, genetic or environmental factors. In general, reduced fertility is a result of one form of reproductive dysfunction, although in more severe cases there can be more than one source of an individual's diagnosed sub- or infertility. There may also be couples that concurrently experience reproductive dysfunction, or even numerous complications that may explain their inability to conceive, which will be discussed further in Chapter 3.

Finally, *sterility* the inability to produce children, often done intentionally through a medical procedure, and is the most popular method of contraception used by couples in the U.S. (Bartz & Greenburg, 2008). Sterilization procedures can be surgical or nonsurgical but serve the same purpose—to prevent reproduction. The most common form of sterilization for women is a tubal ligation, commonly referred to as getting one's "tubes tied." Tubal ligation is an effective method of surgical sterilization that involves blocking or completely removing the fallopian tubes (Bartz & Greenburg, 2008). Another form of surgical sterilization for women is a hysterectomy, or removal of the uterine body. The hysterectomy procedure is not often used for sterilization purposes unless a woman has other chronic medical problems in which a hysterectomy may prove to be more beneficial, for example in severe cases of endometriosis or adenomyosis, uterine fibroids, recurrent uterine prolapse, gynecologic cancer prevention, and other severe reproductive disorders (HHS, 2019a). Hysterectomies can also involve removal of other internal structures of the female reproductive system such as the ovaries, fallopian tubes, or cervix, but is not required. Non-surgical methods of sterilization have been more contentious in the U.S. The nonsurgical Essure procedure used a catheter to insert coils of polyethylene terephthalate into the fallopian tubes that induced fibrosis and eventual tubal occlusion (Hurskainen et al., 2010). Essure was approved by the Food and Drug Administration [FDA] in 2002, marketed as a less invasive and significantly cheaper method of sterilization that was still just as effective as tubal ligation. However, due to many women reporting complications with Essure long-term, the FDA withdrew Essure from the market in 2018 and all unused units were required to be returned (Shuren,

2020). Currently there are no reliable, FDA approved nonsurgical forms of female sterilization.

For men, vasectomies are the longstanding method used for male sterilization. Typically performed under a simple local anesthetic, the conventional vasectomy prevents sperm from entering the urethra during ejaculation by cutting and sealing off the tubes that deliver sperm to seminal fluid—the vas deferens (Bartz & Greenburg, 2008). Over time, several adaptations to the conventional vasectomy have been introduced, with the most popular new technique being the no-scalpel vasectomy [NSV], or "key-hole" vasectomy (Bartz & Greenburg, 2008). The NSV procedure is still as effective as a conventional vasectomy but is less invasive and has a much shorter recovery period. The NSV uses a pinhole split in the scrotum to access the vas deferens and requires no stitches for proper healing, compared to the conventional vasectomy that requires a longer slit and one to two sutures to close the wound that are removed by a urologist several weeks later (Bartz & Greenburg, 2008). Use of a smaller slit shortens healing time, decreases likelihood of infection, and lowers the chance of post-operative complications. Vasectomies are currently the only method of sterilization utilized for men.

Patients who have pursued sterilization typically do not regret their decision to become sterile (Bartz & Greenburg, 2008). Still, there are instances in which patients change their mind later and would like to have children. Depending on the method of sterilization used, it may be possible to undergo a reversal. Vasectomies and tubal ligations can be reversed in an attempt to restore an individual's ability to conceive. However, patients that undergo a sterilization reversal are not guaranteed to regain their

fertility, and the likelihood of having a successful reversal resulting in regained fertility decreases over time—the longer a patient has been sterilized and more invasive their sterilization procedure was, the less likely they are to regain their ability to conceive naturally after a reversal (Bartz & Greenburg, 2008). Some patients may still experience some extent of subfertility, or more extreme cases, infertility even following their reversal. In cases of infertility or prolonged subfertility, couples would then be encouraged to pursue fertility treatment using ARTs to conceive.

Conclusion

The human reproductive system is complex and many timepoints during development can be disrupted leading to dysfunction and resulting in infertility. Individuals experiencing prolonged inability to conceive may seek medical advice to understand the reason why they have been unable to conceive on their own. For patients, physicians and the scientific community, the benefits of understanding the underlying causes of reduced fertility are multifaceted: patients are more informed regarding their personal health, physicians can diagnose and treat patients with various forms of reduced fertility, and the scientific community can use this information to develop novel therapies and treatments to help address reduced fertility. In the following chapter, I characterize the different forms of male-factor and female-factor infertility, as well as factors that can negatively impact fertility regardless of sex.

CHAPTER 3

DIAGNOSIS AND CHARACTERIZATION OF REDUCED FERTILITY

Synopsis

Normal function of the male and female reproductive systems involves numerous complex and dynamic processes. These processes work on both individual and cooperative levels to orchestrate creation and maturation of gametes, movement of gametes through the reproductive system, as well as fertilization and pregnancy. During any step of conception or pregnancy, the entire reproductive process can be terminated by one or more complications: hormone imbalances, genetic anomalies, anatomical abnormalities, infection, or environmental factors (Ford & Schust, 2009). This can manifest as reduced fertility for either sex, or as recurrent pregnancy loss [RPL] for women (Garrisi et al., 2009; Ford & Schust, 2009). For couples with no known reproductive dysfunction who are struggling to start a family naturally, many turn to the counsel of a licensed obstetrician-gynecologist [OBGYN].

New patients at the fertility clinic are required to undergo basic fertility testing to determine the source and severity of their impaired fertility. Fertility testing can reveal numerous conditions affecting an individual's ability to conceive—some of which may be necessary (or potentially beneficial) to address prior to proceeding with fertility care. In this chapter, I cover routine testing and examinations conducted in fertility clinics for both men and women, aimed to elucidate potential sources of reduced fecundity. I learned about each of these tests during my tenure as an andrologist and embryology trainee at the fertility clinic through discussions with the attending nurses and physician that curated treatment plans for patients based on the results of their diagnostic testing.

For each testing parameter, I describe normal/expected ranges and the potential clinical significance of an abnormal result. This chapter is indented to describe how fertility is assessed in the clinic to articulate what is meant by the *pathology of infertility*—specifically what dysfunction is occurring within the human body that gives rise to abnormal results, which necessitate the use of Assisted Reproductive Technologies that will be covered in Chapter 4.

Who is at Fault?

The entire reproductive process is a complex dynamic between two individuals. Dysfunction within the reproductive system is equally complex to diagnose, which can be caused by one or more factors that may influence one another during conception. Sources of reduced fertility can come from one or both partners, and as a result, diagnosing reduced fertility is not always a straightforward process. In some cases, reproductive dysfunction cannot be diagnosed with complete certainty. Idiopathic infertility has been reported to affect roughly 30% of infertile couples worldwide (Sadeghi, 2015). Patients who seek fertility treatment often expect to be told a definitive cause and guaranteed treatment for their reduced fertility—but this is not always the case and is seldom as simple as blood test or ultrasound. The initial attempt to diagnose underling reduced fertility can be a time-consuming, extensive, and invasive process, and for patients who have been trying to conceive for over a year without success, visiting a fertility clinic seldom feels like the "first step" of their family planning.

If treating the underlying condition may improve a patient's capacity to conceive naturally or maintain a pregnancy, the patient is encouraged to pursue treatment of the reproductive dysfunction beforehand (ACOG, 2019). Addressing reproductive

dysfunction through medications and/or medical procedures can push conception off for several months even under the best of circumstances. If the source of reproductive dysfunction is rectified, patients may no longer need to seek care at a fertility clinic. However, recommended treatments are not a guaranteed fix even if they seem promising. If the condition is not treated successfully, this can further delay fertility treatment and pose an even more significant financial burden on patients trying to conceive (ACOG, 2019).

To determine the best path to parenthood for each patient, clinics typically begin with extensive paperwork that patients are required to fill out. These forms ask for information such as: contact information for the patient as well as an emergency contact, insurance information for billing purposes, and personal and family medical history questions. Patient's answers to these questions help physicians determine which forms of diagnostic fertility testing will provide the best information regarding their reproductive health and construct a comprehensive plan for treating each patient.

Male Fertility Testing

Fertility testing for men begins with blood work to check for sexually transmitted infections [STIs] and testosterone levels. As previously discussed, testosterone is a fundamental steroid hormone necessary for the production of sperm (Betts et al., 2013). According to the American Urological Association, in men of reproductive age, total testosterone levels detected through blood work should not fall below 300 nanograms per deciliter (Mulhall, 2018). If total testosterone levels appear to be low, a repeat testosterone measurement will be used to confirm testosterone deficiency which may be a potential cause for male infertility (Surbone et al., 2019). Conversely, high testosterone

levels typically seen in conjunction with testosterone therapy can lead to reduced fecundity, as high levels of testosterone suppress production of FSH (Table 1). The presence of STIs in men has a minor impact on male fertility in Western countries, as STIs are often treated and not left to worsen over time (Ochsendorf, 2008). However, some studies link immunodeficiency with lower sperm quality, often due to the production of antisperm antibodies (Ochsendorf, 2008). Antisperm antibodies are proteins that attach to sperm cells, hindering their movement (Vickram et al., 2019). This impedes the ability of a sperm cell to navigate through the reproductive system and prevents them from being able to fertilize an oocyte (Shibahara et al., 2020). The body recognizes sperm with antisperm antibodies attached as foreign material. The immune system then targets these antibodies and attempts to remove them, which damages or kills the sperm. Immunodeficiency disorders can be genetically inherited through genetic mutations, infections, or exposure to harsh toxins (Vickram et al., 2019). While there are many different symptoms and treatments for immunodeficiency disorders, a compromised immune system can be a cause for reduced fertility (Vickram et al., 2019).

The second component of fertility testing for men is a standard semen analysis which is used to assess the quality and quantity of sperm found in the ejaculate. A sample of semen is provided to the fertility clinic's andrology laboratory where it is evaluated based on standardized criteria. The most widely utilized and accepted set of criteria for semen analyses has been established by the WHO, published in the *Laboratory Manual for the Examination and Processing of Human Semen*. Use of standardized criteria for a semen analysis is important for accurate and uniform diagnoses which can be understood by many clinics rather than a select few (WHO, 2010). The WHO's standards for semen

analyses evaluate the sample using both macroscopic and microscopic parameters. Laboratory technicians are trained to evaluate semen based on these parameters within a reasonable degree of accuracy and reproducibility and conduct these analyses in a certified clinical laboratory setting (see figure 7).

Figure 7. Andrology Laboratory Setting. This is a photo taken of my workspace in the andrology laboratory at the fertility clinic where I worked. Photo Credit: Shelbi Peck, 2019.

However, it is important to bear in mind that many semen analysis parameters are qualitative assessments presented on a spectrum, so there may be intermediate or combinative results. Also, “normal” is a relative term and semen samples vary not only from person to person, but also to a small degree from collection to collection as well. An initial semen analysis with a significant abnormal result or several values that are out of the normal range typically require a repeat analysis to confirm the results. One or two abnormal results with little clinical significance, or borderline normal results may not

impact the clinical diagnosis as they would have little to no effect on the male partner's fertility. In this case it is often the clinician who makes suggestions for a couple's treatment plan based on the overall results of both the male and female partner's fertility testing results.

A semen sample is initially assessed using macroscopic parameters for variables that are considered informative, specifically liquefaction, appearance, volume, viscosity, and pH (WHO, 2010). Liquefaction time is measured first, then the remaining macroscopic observations and measurements are recorded (Baskaran et al., 2020). These parameters will be discussed in detail in Table 3 along with general variations and the potential clinical significance of each. The term "potential clinical significance" is used because an abnormal result is not *always* indicative of reproductive disorder/dysfunction. Repeated abnormal results seen through additional testing are used to confirm a diagnosis of reproductive dysfunction. For example, if low volume is reported for a patient's first semen analysis, this may be indicative of an obstruction of the ejaculatory duct, partial retrograde ejaculation, androgen deficiency, or can merely be a result of undisclosed loss of part of the sample during collection. Patients are asked to document any loss of the sample and specify which part of the sample was lost: first, middle, or last (Baskaran et al., 2020). The first part of the ejaculate is known to have the highest quality and quantity of sperm, so loss of the first part of the sample can negatively affect other parameters of a semen analysis such as total sperm number, concentration, and morphology (Hebles et al., 2014). In the clinic, male patients receive verbal and written instructions that state they must collect a complete sample and note any sample loss. They are likely unaware of the importance of collecting a complete sample, and that loss of the first portion of the

ejaculate in particular may have a significant impact on the results of the analysis. Reporting a poor collection can also be embarrassing, so patients may not be forthcoming with laboratory staff about losing of a portion of the sample. Therefore, in this case and for most other instances of poor or abnormal results, a repeat semen analysis is important for determining if the counts observed are typical for that patient or were a result of a poor collection that day.

It is important to keep in mind that patients who are visiting a fertility center are often there because they are struggling to conceive naturally and will likely require some degree of fertility treatment. Taking this into account, abnormal results for semen analyses are anticipated in a fertility clinic setting. Values that are slightly out of range may not require treatment if the patient intends to use an ART to conceive. However, male patients with recurring severe abnormal values may be recommended to follow up with a urologist who can more confidently diagnose and treat urogenital conditions.

Following completion of the macroscopic analysis, a more thorough observation of the sperm inside of the ejaculate is conducted through a microscopic analysis, where a small aliquot of unstained semen is observed under a phase-contrast microscope. Microscopic analysis of the sample can reveal qualities such as: mucus strand formation, agglutination, round cells, sperm motility, count, and morphology (WHO, 2010). The sample is gently but thoroughly mixed using a pipette to homogenize the semen. This ensures that a representative aliquot is taken from the sample for assessment. Several tools can be utilized for conducting a microscopic analysis on a semen sample; the aliquot can be mounted on a glass slide covered by a thin coverslip, loaded into a disposable hemocytometer counting chamber, or onto a washable Makler Counting

Chamber. Regardless of the technique used, a standardized volume of semen, typically 10µl is loaded into the counting apparatus. Table 4 outlines the standard microscopic aspects of a semen analysis, and similar to Table 3, shows general variations and the potential clinical significance of each.

Both macroscopic and microscopic evaluations of semen give comprehensive insight into male fertility and contribute to a couple's fertility treatment plan (Baskaran et al., 2020). Abnormalities in a semen analysis can reveal reproductive dysfunction on a larger scale—anatomical abnormalities, obstruction in the reproductive system, infection, or complications with the immune system, which will be discussed in section 2. The process of diagnosing reproductive dysfunction in men is much shorter than it is for women. This is due to male-factor infertility testing being less invasive and requiring fewer tests in general, compared to the elaborate regimen of testing performed to diagnose female infertility. The effects of male infertility play a more detrimental role early in the scope of reproduction—fertilization is impacted, but upon successful conception, male reproductive dysfunction does not impact the pregnancy further.

My training as an andrology laboratory technician at the clinic began with understanding male fertility testing. First, I was required to conduct side-by-side semen analyses with a senior andrologist to ensure that my ability to analyze sperm motility, progression, count, and morphology was accurate and consistent with the other laboratory staff. My training required 40 side-by-side semen analyses with accurate results before I received approval to conduct analyses independently and report results to patients. After becoming proficient in conducting semen analyses, I was tasked with understanding the results of a semen analysis and the clinical significance that abnormal results may have—

such as azoospermia potentially due to use of testosterone, or antibiotic use and high round cell count. Understanding the techniques and clinical significance of the results was extremely valuable and gave me insight into the pathology of male infertility.

Male-Factor Infertility

There are numerous conditions and factors that can influence fertility potential in men. The results of a standard semen analysis are often utilized as the first tool for determining reproductive capacity of male patients. Data analyses on the distribution of the source of reduced fertility by sex have shown that male, female, and either unexplained or reduced fertility in both sexes represent equal proportions of the infertility cases reported in the U.S. (Chandra et al., 2013). Determining which partner(s) have reduced fecundity as well as the source and severity of their condition or disorder are crucial steps for formulating a plan for conception. Some of these conditions are irreparable, meaning some form of use of ART will be necessary to conceive, while others may require preemptive treatment to correct or abate symptoms. The Eunice Kennedy Shriver National Institute of Child Health and Human Development [NICHD] categorizes sources of reduced male fertility into two distinct groups: conditions that affect how sperm is made and conditions that affect the way sperm is transported (NICHD, 2017).

As discussed in chapter 2, spermatogenesis is a complex, cyclic process which under normal circumstances results in mature spermatids with a haploid genome—only one set of chromosomes (Betts et al., 2013). Errors and complications during any step of spermatogenesis can lead to chromosomal defects, morphological deformities, and other abnormal semen pathologies such as asthenozoospermia, ologizoospermia and

necrozoospermia. There are numerous underlying issues that can cause defective spermatogenesis, but according to Mayo Clinic, the most common reversible cause of male factor infertility is a varicocele (Mayo Clinic, 2018). Varicoceles are enlarged veins in the scrotum surrounding the testicles. While there is currently no consensus regarding the exact reason why varicoceles and infertility are so often seen together, there have been proposed theories involving hyperthermia, blood flow, buildup of reactive oxygen species, and hormonal imbalances (Agarwal et al., 2006; Lipshultz & Eisenberg, 2011). Patients with one or more varicoceles often exhibit poor semen parameters for morphology and sperm count. Varicoceles can be repaired through an outpatient microsurgical procedure called a varicocelectomy (Lipshultz & Eisenberg, 2011). Pending no complications during recovery or additional conditions impairing fecundity, a successful varicocelectomy restores normal blood flow to the testes and normal sperm production can resume.

In some men, the testes themselves may not have descended fully during fetal development. When the testes are still housed within the abdominal cavity, they are exposed to excessive heat, which inhibits spermatogenesis and leads to azoospermia or oligozoospermia (Minuto et al., 2011). Clinically this condition is referred to as cryptorchidism and is present in roughly 3% of full-term and nearly 30% of preterm male infants (Minuto et al., 2011). The testes can be surgically relocated from the abdomen into the scrotum to help prevent pain and reduced fertility later in life, but likelihood of a successful testicular relocation to restore fertility is low, especially if this condition remains unaddressed into adulthood. Even if the testes descend properly during development, other problems with the testes such as testicular trauma or swelling due to

infections may also lead to reduced fertility (Redmond et al., 2018). Testicular trauma is most likely to occur during contact sports but can be caused by other severe accidents as well. Bruising of the scrotum is the most common side effect of testicular trauma and can resolve itself naturally—but heavy bleeding within the scrotum, termed scrotal hematoma, is more severe and can require surgery (Mayo Clinic, 2020b). Trauma to the testes can negatively impact the production of testosterone and hinder production of sperm (Redmond et al., 2018). Swelling of the testes due to infection can also have similar effects on spermatogenesis (Trojian et al., 2009). Both bacterial and viral infections of the testes can lead to swelling, although both forms of infection can be treated within a matter of a few weeks. Sexually transmitted infections are the leading cause of bacterial orchitis, which is treated through prescribed antibiotics (Trojian et al., 2009). However, if any form of testicular infection is left untreated, patients may experience pain, discomfort and ultimately a drop in production of testosterone.

Any condition that impacts the way hormones are produced, utilized, or stored within the body can affect male fertility. Spermatogenesis is under tight hormonal regulation, and excessive (or insufficient) levels of even one hormone circulating in the body can disrupt production of sperm. Just as testicular trauma can cause low levels of testosterone which slows spermatogenesis, there are conditions in which high levels of hormones can negatively impact sperm production. Hyperprolactinemia is a thyroid condition characterized by an abnormally high presence of prolactin in the bloodstream. Prolactin inhibits secretion of GnRH from the hypothalamus, which then inhibits production of other hormones necessary for spermatogenesis such as FSH, LH and testosterone (De Rosa et al., 2003). Normally, levels of each hormone rise and fall in a

cyclic pattern throughout spermatogenesis, but in cases of hyperprolactinemia, sperm production is arrested, resulting in low sperm count, decreased sperm motility and low ejaculate volume (De Rosa et al., 2003). In some cases, side effects of hypo- and hyperthyroidism can be remedied—for instance, hyperthyroidism brought on by a treatable condition such as varicoceles can be resolved through a successful varicocelectomy (Lipshultz & Eisenberg, 2011). However, not all cases of hypo- and hyperthyroidism can be resolved—nearly all other thyroid diseases cannot be cured, their symptoms can only be managed. Patients with preexisting thyroid disorders unrelated to reproductive dysfunction may require variations in treatment. Understanding a patient's full personal and family medical history provides the physician with the most comprehensive picture of overall health, which ultimately affects the reproductive system. Patients may not be fully aware of the extent that different organ systems contribute to overall healthy reproductive function and may need to be prompted with more specific questions during a consultation. Rather than relying solely on a new patient information form to guide a consult, it is helpful to expound upon the medical history component to discuss with the patient how reproductive health can be viewed as a reflection of overall health. Informative conversations with specific questions can help the patient more thoroughly evaluate their personal and family medical history which makes for more helpful consultations.

The second category of conditions that can cause reduced male fertility are those related to the transport of sperm. The ejaculatory process is largely reliant on two factors: production of a sufficient volume of seminal fluid to carry the sperm, and unimpaired reproductive ducts that are free of damage or blockage so sperm can pass through readily.

In table 3, the macroscopic parameters of a standard semen analysis are listed along with their respective ranges and significance. The normal ejaculate volume ranges between 1.5-5.5mL, with any volume below 1.5mL classifying as abnormal. Low or no ejaculate volume (anejaculation) can be caused by several different conditions and presents a significant barrier to proper fertilization (WHO, 2010). Chapter 2 provided a sequence of semen production, transport out of the male body, and through the female reproductive system to the point of fertilization of an ovulated oocyte. Throughout this process, many of the sperm are filtered out, specifically, those that are immotile or have low motility and sperm that are abnormally shaped. By the time the sperm reach the fallopian tube, only the most promising, healthy sperm remain (Suarez & Pacey, 2005; Betts et al., 2013). If the semen volume is low, the number of sperm trying to reach the oocyte will often be lower, decreasing the likelihood that any sperm will make it to the oocyte in time for fertilization (WHO, 2010). The most widespread cause of low semen volume is due to utilization of different medications--specifically antidepressants (Brody & Gu, 2020). In a data briefing published by the CDC's National Center for Health Statistics, between 2015-2018 approximately 13.2% of adults in the U.S. had used antidepressant medications within the past month (CDC, 2020). In regard to fertility, antidepressants are most commonly associated with causing low sperm count, decreased motility and low semen volume in men (Beeder & Samplaski, 2019). There are several classes of antidepressant medications, which all affect semen parameters in different ways. Most medications for anxiety and depression work to inhibit reuptake of neurotransmitters in the brain, but these changes within the chemistry of the brain are speculated to impact endocrine function as well (Koyuncu et al., 2012). Manipulation of hormone production

can hinder secretion of seminal fluid from the seminal vesicles and prostate, resulting in low semen volume, or no ejaculate volume at all (Beeder & Samplaski, 2019). Beeder and Samplaski's study in 2019 concluded that discontinuing use of antidepressants can allow for normal regulation of the reproductive system. Within a month, some patients reported normal semen analyses following cessation of antidepressant use. However, despite promising improvements in semen analysis parameters when antidepressants are not influencing hormonal regulation, this may not be feasible for all patients (Beeder & Samplaski, 2019). Other medications such as antifungals, painkillers, blood pressure medications and chemotherapy medications can alter hormone synthesis and utilization in the body, which can also impact reproductive function (Beeder & Samplaski, 2019).

Retrograde ejaculation is a condition influenced by improper hormonal regulation in the body that can result in low or no semen volume. Related to anejaculation, retrograde ejaculation is caused by incomplete constriction of the muscular sphincter that contracts during ejaculation to prevent backflow of semen into the bladder—causing semen to enter the bladder and mix with urine (Mayo Clinic, 2019a). Retrograde ejaculation is sometimes associated with nerve damage or surgery on the bladder or prostate but is most commonly seen as a side effect of certain blood pressure medications and antidepressants (Mayo Clinic, 2019a). Semen mixing with urine in the bladder is not toxic or harmful to the body but does lead to infertility and may require use of ART to conceive so sperm can be retrieved from the urine and washed before use. In cases where specific medications may be causing reduced male fertility, patients are typically recommended to consult the physician who prescribed them the medication to discuss a

plan: a temporary change in dosage, complete discontinuation, or no change in the prescription can be made.

Hormonal regulation is integral to many physiological aspects necessary for the transport of sperm during ejaculation (Betts et al., 2013). Sexual arousal in men that leads to an erection is a complex process that requires feedback from numerous body systems: endocrine, neural, and cardiac. Malfunction in any of those systems can lead to erectile dysfunction—a relatively common disorder characterized by inability to get or maintain an erection (Mayo Clinic, 2020a). Erectile dysfunction is associated with reduced fertility because ejaculation becomes improbable when there is not enough blood flow to the penis to facilitate ejaculation and expulsion of semen (Mayo Clinic, 2020a). Medications, health conditions, injuries, and lifestyle choices such as heavy drinking or smoking often contribute to the development of erectile dysfunction (Razdan et al., 2017). While occasional erectile dysfunction is not typically a reason for concern, persistent erectile dysfunction can cause reduced fertility and is also known to cause psychological effects (Mayo Clinic, 2020a). Treatment of this condition must be approached on a case-by-case basis. Medications such as Cialis, Viagra, and Stendra help to encourage erections through trapping more blood in the penis but may not be suitable for patients with underlying medical conditions related to cardiovascular health. Patients with heart disease, high blood pressure, diabetes, or high cholesterol may need to seek an alternative form of treatment if medications for erectile dysfunction may negatively interact with their current prescribed medications (Razdan et al., 2017). Erectile dysfunction is a complex yet treatable condition that can be managed or resolved starting with a proper diagnosis (Turek et al., 2018).

Obstruction can also be a cause of male infertility. Ejaculatory duct obstruction, or EDO, is a rare cause of several abnormally low semen parameters that can be a result of genetic factors, trauma, infection, or formation of cysts within the ejaculatory pathway. EDO is a physical blockage which does not impact hormonal regulation in the body—therefore diagnosis is confirmed through an ultrasound rather than a blood test. There are three types of EDO: partial, complete, and functional (Modgil et al., 2015). A "partial obstruction" is incomplete but still impacts semen analysis parameters—semen volume is low but there are some motile sperm present in the ejaculate. Complete obstruction is the most severe case where expulsion of sperm is blocked, resulting in low volume and azoospermia (Modgil et al., 2015; Lawler et al., 2006). The last type of obstruction is a functional obstruction. Functional obstructions signify suboptimal transport of semen into the urethra, but ultrasounds reveal no complete or partial blockage (Modgil et al., 2015). Patients typically seek medical advice after repeated low volume of semen or pain during ejaculation. Depending on the results of the semen analysis, the physician can rule out or identify EDO as a source of infertility and use an ultrasound to confirm the extent of the obstruction (Modgil et al., 2015).

There are three possible treatment routes for patients with obstructions in the ejaculatory duct: medication, surgery, or sperm aspiration. Medications may be able to stimulate normal function in cases of functional obstruction, although cases of partial or complete obstruction will not likely benefit from medication alone. Partial or complete obstructions are better addressed through transurethral resection of the ejaculatory duct [TURED]—a process that reopens the ejaculatory duct and removes any blockages preventing passage of semen (Mekhaimar et al., 2020). The TURED procedure is

relatively invasive and has numerous potential complications during and after surgery, so this surgery is recommended for more severe cases or when the patient is experiencing pain related to the obstruction (Mekhaimar et al., 2020). The last method for overcoming EDO is sperm aspiration (Jarow, 1994). Since EDO does not impact production of sperm, only delivery, mature sperm can be retrieved through a testicular sperm extraction [TESE]. The TESE procedure uses a small needle to extract some of the seminiferous tubules in the testicle, after which the tubules can be teased apart so sperm can be removed. TESEs have fewer postoperative complications than the TURED procedure but is not a fix for EDO—just a way around it to conceive (Shah, 2011; Mekhaimar et al., 2020). Patients who would like to fully resolve their EDO for quality of life or to improve chances of natural conception would be encouraged to pursue TURED, but patients using ART to conceive can use either procedure to improve their chances of successful conception (Mekhaimar et al., 2020).

There is a final category of male infertility that can cause issues with both production and transport of sperm, so it merits being discussed on its own. Congenital abnormalities of the reproductive system and other genetic causes of infertility have diverse effects on the male reproductive health. Testicular dysgenesis syndrome is a largely gene-related disorder that is characterized by cryptorchidism, hypospadias, and testicular cancer (Skakkebæk et al., 2001). Cryptorchidism, as discussed previously, is a condition where one or both testes have not descended into the scrotum during fetal development, leading to poor quality of semen and decreased sperm production (Minuto et al., 2011). Patients with cryptorchidism are also more likely to be diagnosed with testicular cancer later in life (Cheng et al., 2018). The last condition associated with

testicular dysgenesis syndrome is hypospadias which is characterized by abnormal location of the urethral opening of the penis (Thorup et al., 2010). Rather than exiting through the top of the penis, in patients with hypospadias, both urine and semen exit through distal portion of the penis: the underside of the corona, along the midshaft, or where the shaft meets the perineal raphe of the scrotum. The symptoms of testicular dysgenesis are associated with mutations in androgen receptors that contribute to development of the penis and testes (Thorup et al., 2010). Both cryptorchidism and hypospadias can be corrected surgically, but treatment of testicular cancer is more complex and may require a combination of surgery, radiation therapy and chemotherapy which can have severe impacts on fertility.

Genetic abnormalities and abnormal sexual development can also lead to absence of male reproductive structures altogether such as anorchidism, penile agenesis, congenital bilateral absence of the vas deferens and persistent Mullerian duct syndrome. Each structure of the male reproductive system plays an essential functional role in fertility and absence of any structure can lead to severe infertility. Anorchidism is a rare testicular disorder that is different from congenital cryptorchidism—despite having a normal XY karyotype, in anorchic patients, the testes failed to develop properly during fetal development and regressed after the critical gonadal determination time period, leading to no testes being present at birth. Closely related to anorchidism is penile agenesis—absence of the penis at birth in a child with an XY karyotype. Anorchidism and penile agenesis can occur independently or in conjunction, but in cases of concurrent disorders, penile agenesis often manifests as micropenis rather than complete agenesis. Occurrence of anorchidism is significantly more common than penile agenesis, with

unilateral anorchidism affecting 1 in 5000 males and bilateral anorchidism affecting roughly 1 in 20000 males (Melmed et al., 2016). Testicular implants are available as a cosmetic surgery to help improve body image, but there are no treatments that will allow for restoration of fertility if both testes are absent, or the one testis present (unilateral anorchidism) does not function properly. Penile agenesis affects only 1 in 30 million births and can be corrected through phalloplasty and urethroplasty—although there are very few urologists qualified to perform these procedures (Melmed et al., 2016; Castro et al., 2007).

Congenital absence of the vas deferens (unilateral and bilateral) [CAVD] is a rare sexual disorder that is reported to occur in roughly 1-2% of men that experience infertility (Hussein et al., 2011). CAVD is caused by a genetic mutation in the *CFTR* gene, which is most commonly associated with cystic fibrosis. A study conducted in 1995 showed that 95% of male patients with cystic fibrosis subsequently had CAVD as well (Chillón et al., 1995). Without the vas deferens, the ejaculate will not contain any sperm and the patient will be diagnosed with azoospermia (Bieth et al., 2020). There are currently no treatments for CAVD to restore normal reproductive potential for patients, although sperm can be retrieved from the epididymis via a percutaneous epididymal sperm aspiration [PESA], or through a TESE during infertility treatment (Shah, 2011). The final noteworthy genetic condition that can affect male fertility is Persistent Mullerian Duct Syndrome [PMDS]. During early embryonic development of genetically male embryos, the Mullerian duct degrades and the Wolffian duct persists to give rise to the structures of the male reproductive system (Ortega et al., 2018). PMDS is a congenital autosomal recessive disorder that affects the way anti-Mullerian hormone is

produced and utilized in the body (Picard et al., 2017). Insufficient levels of anti-Mullerian hormone during development of the reproductive system causes the Mullerian duct to persist rather than degrade, leaving the embryo with a mixture of both male reproductive structures some primitive female reproductive structures (Betts et al, 2013; Ortega et al., 2018). The most common condition associated with PMDS is cryptorchidism—the testes were unable to descend because a primitive uterus developed as well, blocking the testes from descending to the scrotum (Picard et al., 2017). A hysterectomy can be performed to remove the uterus to allow for relocation of the testes. This surgery is precarious due to these reproductive structures adhering to one another during development and is ideally performed prior to puberty but can also be performed later in life (Sherwani et al., 2014).

Infertility is a multifactorial disorder that can be a result of one or a combination of anatomical, physiological, and genetic conditions. In addition to these factors, there are also environmental and lifestyle factors that contribute to an individual's reproductive capacity as well. These factors influence both sexes in different ways and will be discussed in section 3.7: Risk Factors Impacting Both Men and Women.

Female Fertility Testing

Maternal health plays a much more significant and long-lasting role in the reproductive process, despite the seemingly equal contributions of the male and female reproductive systems during conception. A hormone imbalance in women can cause ovulatory dysfunction, irregular menstrual cycles, and even spontaneous abortion well into gestation. The additional complexities and contributions of female anatomy during

reproduction, pregnancy, and birth make diagnosing and treating reproductive disorders in females a more convoluted process.

In light of this, fertility testing for women is significantly more extensive and invasive. The standard format for new patient fertility testing begins with a consultation, pelvic exam, a full panel of bloodwork to assess baseline hormone values, a sonohysterogram and a hysterosalpingogram. Bloodwork is conducted on a routine basis to track hormone cycling, and additional tests may be required if the patient has abnormal bloodwork or exams. The additional complexities and long-term contributions of the female reproductive system call for a more thorough testing regimen. Consultation with an OBGYN establishes the patient's basic medical history, including known medical conditions, medications, drug/alcohol use, and any exposure to environmental toxins or radiation. The patient is also asked about her sexual history: use and method(s) of birth control, past pregnancies and/or miscarriages, information about her menstrual cycle, history of sexually transmitted infections [STI's], pain or discomfort during sexual intercourse and frequency of intercourse.

Following completion of the consultation, the physician conducts a pelvic exam on the patient to assess general reproductive health through an external and internal visual exam (Mayo Clinic, 2019c). The patient's vulva is inspected first for any signs of redness or irritation, and to ensure there is no abnormal discharge coming from the vagina (Mayo Clinic, 2019c). Then, the physician inserts a vaginal speculum tool into the vagina so the vaginal walls and cervix can be examined for any abnormalities. At this point, the physician can decide whether the patient needs a Papanicolaou test or *pap smear*, which uses a swab to collect cells from the cervix to check for cervical cancer

(Mayo Clinic, 2019c). According to the American College of Obstetricians and Gynecologists, cervical cancer is primarily caused by infection with Human Papillomavirus (ACOG, 2016). While most women in low-risk groups have normal pap smears every 3-5 years, an abnormal pap smear will require treatment which takes precedence over addressing infertility (Arbyn et al., 2010). If cervical cancer is caught at an early stage, the patient may be recommended to freeze her eggs for future use with a surrogate, although later more aggressive stages may need to be treated immediately, with no time for the patient to pursue fertility preservation (Inhorn et al., 2013). Treatment of cervical cancer varies depending on the severity of the case, but potential treatment options include but are not limited to hysterectomy and/or use of chemotherapy—both of which would cause permanent infertility, making normal cervical health an important component of fertility treatment and general health screening.

The next step of fertility assessment for women is conducting a blood serum assay of female reproductive hormones and molecules. The roles of many of these hormones and molecules within the female reproductive system have been discussed in Table 2. While these elements are present in the female body simultaneously, quantities of each molecule found in blood serum vary throughout the menstrual cycle. An initial panel is typically conducted on day 3 of the patient's menstrual cycle which include: estradiol, FSH, LH, progesterone, prolactin, Anti-Mullerian Hormone [AMH], Thyroid Stimulating Hormone [TSH], Free/Total Thyroxine [T4] and hCG. A follow up blood draw of progesterone is conducted on day 21—assuming the patient follows the typical 28-day menstrual cycle. In cases of abnormal menses, the OBGYN will adjust the timing of blood draws that most closely mimics the patient's individual cycle. By re-quantifying

these values at both the beginning and end of the cycle, the physician can gain a more comprehensive understanding of hormone cycling in that patient to establish a baseline. Table 5 demonstrates normal assay values for each hormone and molecule by cycle day, if applicable. The “Result” column also describes potential implications of low/high values for each—though some abnormal results may appear simultaneously, as production of these molecules is tightly regulated by positive and feedback with other molecules and organ systems. Ranges for each molecule listed in Table 5 are reflective of healthy, unstimulated patients—"unstimulated” meaning the patient is not taking any form of oral or injectable fertility medication. Under the influence of ovulation induction medications, blood serum assay values may not always fall within the ranges listed below.

Blood serum assays are invaluable for tracking a patient’s individual menstrual cycle and for discerning whether hormonal imbalances may be an underlying cause of subfertility. For patients seeking treatment at a fertility clinic, bloodwork becomes an integral component for monitoring a patient’s receptivity to fertility medications during their treatment. Although clinics often utilize different systems to quantify the presence of these hormones and molecules in blood serum, the reported normal ranges are standardized so results can be understood from clinic to clinic. Automated immunoassay equipment used in fertility clinic laboratories are calibrated for each molecule and are routinely re-calibrated to ensure accurate readings over time. Quality control tests are also performed daily to ensure that the calibrations are still precise.

After conducting thorough bloodwork to analyze the essential molecular mechanisms regulating the menstrual cycle, the next step is to examine the internal

reproductive anatomy more closely. Information gathered via a pelvic exam is limited—which makes the sonohysterogram and hysterosalpingogram important methods for observing internal reproductive health (Mayo Clinic, 2019c). The sonohysterogram is a procedure used to evaluate the shape, depth, and overall health of the uterine cavity. A catheter is used to deliver a saline solution into the uterine body which causes distension of the uterus (Mayo Clinic, 2019c). The physician observes the appearance of the uterine lining using a transvaginal ultrasound probe in order to determine if there is scar tissue present, uterine polyps, or fibroids that would adversely affect fertility and a woman's ability to carry a pregnancy to full-term (Mayo Clinic, 2019c). Assessing the health of the uterus is important for both conception and pregnancy, but a sonohysterogram alone is often not sufficient to develop a complete picture of a patient's reproductive health. A hysterosalpingogram [HSG] is a procedure utilized to take an x-ray of the uterus and fallopian tubes (Mayo Clinic, 2019c). Like the sonohysterogram, uterine abnormalities can also be observed through an HSG. However, being able to ensure that the fallopian tubes are unobstructed is unique to the HSG procedure. A catheter is passed through the cervix into the uterus where an iodine-based dye is injected into the uterus to create contrast on the x-ray (Mayo Clinic, 2019c). If the dye is unable to pass through the fallopian tube towards the ovary, this signifies a possible tubal obstruction, which will be discussed in the following section titled "Female-Factor Infertility."

At the clinic where I worked, they provided care for between 40-60 patients each month. Most of these patients lived in Arizona and had all their fertility testing performed in-house—during the diagnostic phase as well as during treatment. Blood serum immunoassays were typically the first method of fertility testing. The initial panel of

bloodwork included the following analytes: Estradiol, Progesterone, FSH, LH, PRL, TSH & T4. Initial panels were always conducted on day 3 of the patient's menstrual cycle—which meant that some patients had their initial consult and bloodwork performed during separate appointments. Conducting an initial bloodwork panel is an imperative first step to understanding reduced fertility and preparing for fertility treatment. The initial panel establishes baseline values for each individual patient. This allows the clinic staff to observe patient-specific data on their responses to fertility medications over time. As an andrology laboratory technician, I ran patient's samples for one or more analytes ordered by the physician and recorded the results in the patient's chart using our Electronic Health Record system, DrChrono. Diagnostic sonohysterogram and hysterosalpingogram images were collected by the on-site sonographer.

Visits to the clinic for diagnostic testing mark the very beginning of a patient's treatment. The process of fertility testing can be time consuming and even uncomfortable for many patients—both physically and psychologically. These tests may reveal one or more sources of reduced fertility, or can uncover no discernable cause, called "unknown infertility." I noticed a trend that many couples seeking treatment at the clinic wanted to pinpoint the exact blame for their infertility—either on a part of their own body (hormones, ovarian dysfunction, uterine dysfunction) or on their partner. Due to the many intricacies of female reproductive anatomy and physiology, reduced fertility can take many different forms and can sometimes be difficult to uncover one definitive cause of reduced fertility. Working at the clinic, I became very comfortable with the "hard conversations" about reduced fertility and infertility. However, for patients, accepting this diagnosis as their "reproductive reality" was not always as easy.

Female-Factor Infertility

Reduced fertility in women can be broken down into four different categories: ovarian, fallopian tube, uterine and cervical infertility. In each area of the female reproductive system there are anatomical, endocrine, and genetic elements that can all equally influence reproductive potential. The leading cause of reduced fertility in women is related to ovulatory dysfunction (Mayo Clinic, 2019d). Ovulatory dysfunction is a broad term that encompasses Polycystic Ovarian Syndrome [PCOS], as well as thyroid and adrenal dysfunction (Mayo Clinic, 2019d). Problems with ovulation can cause irregular or missed menstrual cycles, making natural conception more challenging and in some cases, impossible.

PCOS is the most common form of ovulatory dysfunction affecting 5-10% of reproductive age women and is characterized by both anovulatory cycles and the presence of numerous cysts within the ovary observed via ultrasound (Palomba et al., 2009). This condition is confirmed through a transvaginal ultrasound of the ovaries to check for the presence of cysts, but cysts are not always indicative of PCOS (Palomba et al., 2009). Ovaries can naturally develop small functional cysts within a single follicle or the corpus luteum during the menstrual cycle, which are most often harmless, do not cause the patient any pain and disappear without treatment (see Figure 8). Although cysts unrelated to the menstrual cycle such as dermoid cysts and cystadenomas can be larger and more painful and lead to reduced blood flow or ovarian torsion which may require surgical removal (Murakami et al., 2013). The size and quantity of cysts present are observed during a transvaginal ultrasound, followed by bloodwork to confirm PCOS.

Blood serum assays conducted to confirm a diagnosis of PCOS often include FSH/LH, Dehydroepiandrosterone [DHEA]/testosterone, a thyroid panel and prolactin test (Laven et al., 2002).

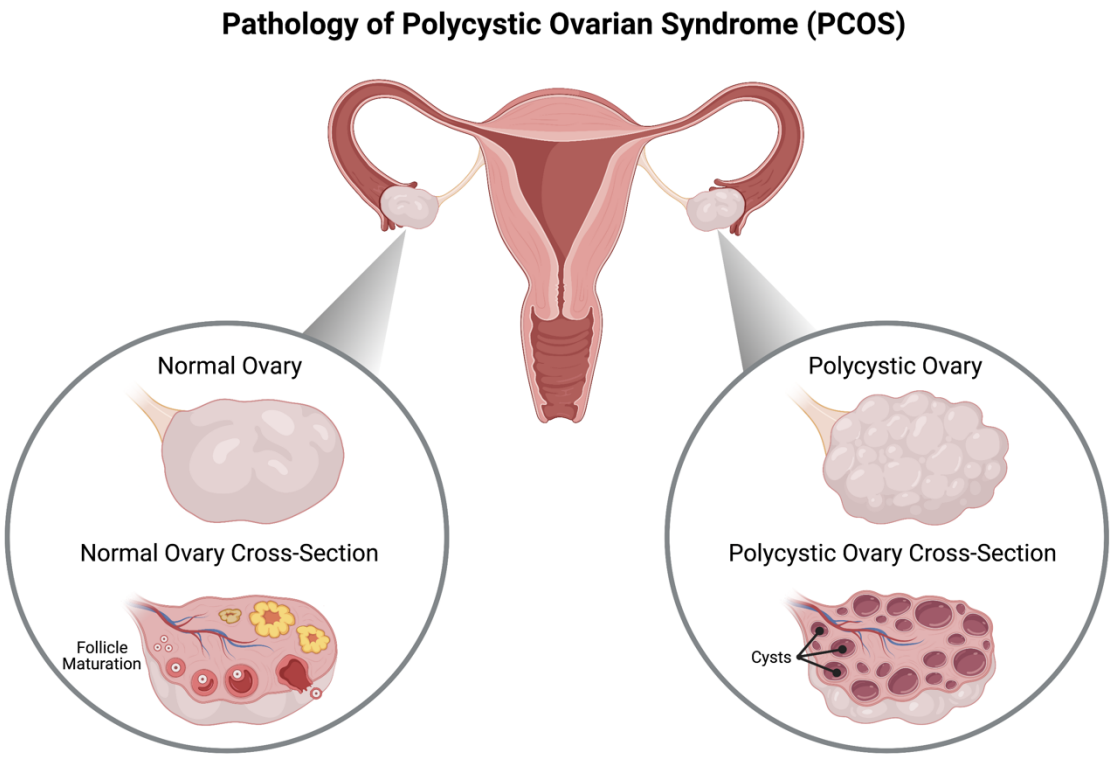

Figure 8. Pathology of Polycystic Ovarian Syndrome (PCOS). This diagram shows the external and internal differences between normal and polycystic ovaries. Created with BioRender.com

FSH and LH are integral hormones for regulation of ovulation and a persistent imbalance throughout the 28-day menstrual cycle can be indicative of ovulatory dysfunction. DHEA and testosterone are both androgens that are present in greater quantities in males but can cause irregular menstrual cycles in women if these molecules are circulating in the bloodstream, as they disrupt normal ovulatory function. Normal

thyroid and pituitary function also play a significant role in the menstrual cycle. Dysfunction of the production or metabolism of TSH, free/total T4 and prolactin can negatively impact the menstrual cycle due to the regulatory impact these hormones have on ovulation. Abnormal levels of TSH and free/total T4 may lead to a diagnosis of hypo- or hyperthyroidism, and high levels of prolactin in the bloodstream (hyperprolactinemia) can impede ovulatory function (Mayo Clinic Laboratories, n.d.-e). Addressing potential hormonal imbalances through thyroid medications or pharmaceuticals to treat hyperprolactinemia can be used to induce ovulatory cycles (Guzick, 2007). Overweight or obese patients who are experiencing anovulatory cycles that may have PCOS are also encouraged to lose weight, if possible, as weight and the presence of excessive amounts of fat tissue can alter ovulatory cycles. These mechanisms of symptom management are considered the first logical steps towards treating infertility potentially caused by PCOS (Guzick, 2007). Especially in the case of unmanaged hypo-/hyperthyroidism and hyperprolactinemia, unaddressed hormonal imbalances can put PCOS patients at risk of ovarian hyperstimulation syndrome during the traditional ovulation induction protocol used for stimulation for intrauterine insemination or IVF (Mayo Clinic Laboratories, n.d.-e).

The same set of blood serum tests can also be utilized to diagnose a second ovulatory disorder called Primary Ovarian Insufficiency [POI]. Synonymous with Premature Ovarian Failure, this condition is characterized by a partial or complete loss of oocytes in the ovary before the age of 40 which causes anovulatory cycles and premature onset of menopause (Santoro & Cooper, 2016). POI can be induced by chromosomal defects such as Turner and fragile X syndrome, exposure to reproductive teratogens, a

result of an underlying autoimmune disease or can be idiopathic (Mayo Clinic, 2019b). Depending on the severity of POI, patients may be able to ovulate sporadically using standard ovulation induction protocols with low success or may resort to using an oocyte donor to conceive if they have no oocytes remaining. Patients with POI typically experience the normal symptoms of the onset of menopause: hot flashes, dry skin, irregular or absent menstrual periods, and vaginal dryness (Santoro & Cooper, 2016). As with blood serum assays conducted to diagnose PCOS, patients with probable POI have their FSH & LH levels tested. Repeated results of abnormally high FSH and LH in the bloodstream can be indicative of POI (Mayo Clinic, 2019b). Mayo Clinic also reported that other important blood serum tests for possible cases of POI include prolactin and AMH. Prolactin levels evaluate for anovulation related to hyperprolactinemia, while low levels of AMH may indicate low follicle count and subsequently suggest POI, but further validation is required (Mayo Clinic, 2019b). Due to the similarities in both blood serum panels and expected results used to diagnose both PCOS and POI, this stresses the importance of conducting a multifaceted analysis. Relying on hormone testing alone can lead to misdiagnosis and improper treatment. Validation of POI requires a transvaginal ultrasound to reveal the number and size of follicles in the ovary, if any are present at all (Baker, 2011). If the ultrasound reveals no follicles or only a few small follicles, POI can be confirmed.

Successful ovulation is the first step towards natural conception—although there are many other barriers to successful fertilization and implantation. Tubal factor infertility can prevent movement of the ovulated oocyte towards the body of the uterus and create a barrier for sperm to reach the oocyte. Examination of the fallopian tubes is

commonplace in an initial fertility workup—typically conducted by an HSG. The HSG exam tracks the flow of radiographic dye through the fallopian tubes and reveals any tubal occlusions present. Obstruction of one or both fallopian tubes is most often caused by an untreated bacterial infection. The most frequently reported form of sexually transmitted infection caused by bacteria is Chlamydia, and if left untreated can lead to pelvic inflammatory disease and tubal factor infertility (Briceag et al., 2015; CDC, 2016). One of the side effects of Chlamydial infection is the formation of a hydrosalpinx—a fluid-filled blockage in the fallopian tube that must be surgically removed (Gorwitz et al., 2017). Removing a hydrosalpinx can be accomplished through a procedure called a tuboplasty where patency is restored, making natural conception possible again (Grisaru et al., 1996). Obstruction can also be caused by pelvic adhesions or scar tissue found on or near the fallopian tubes or ovaries. Pelvic adhesions can cause torsion of the fallopian tubes that obstruct movement of the oocyte but can be removed surgically through laparoscopy (Robertson et al., 2017). Infertility due to tubal obstruction can be bypassed using IVF. However, IVF is not a true solution for tubal-factor infertility.

The largest category of sources of reduced fertility in women is related to uterine pathology, which can impose detrimental effects on both implantation of the embryo and healthy fetal development. The uterus, or womb, is a hollow, pear-shaped muscular organ that is the largest structure in the female reproductive system. Congenital abnormalities of the uterus can lead to amenorrhea, higher rates of miscarriages, and infertility (Chan et al., 2011). The most severe form of congenital uterine malformation is Mullerian agenesis—complete absence of the uterus with partial or incomplete development of the vaginal canal (Nakhal & Creighton, 2012). Mullerian agenesis is caused by a mutation in

the Wnt4 gene which controls suppression of androgen production. Loss of function of the Wnt4 gene leads to the presence of excess androgens that inhibit normal development of female reproductive structures in utero (Sultan et al., 2009). Mullerian agenesis affects 1 in every 4,500-5,000 women and often remains undiagnosed until pubertal age when women experience amenorrhea, as Mullerian agenesis does not inhibit development of secondary sex characteristics (ACOG, 2018). Currently, options for conception for patients with Mullerian agenesis are to pursue both IVF and a gestational carrier. Other anomalies in uterine development include septate, unicornuate and bicornuate uterus, and uterine didelphys (Robbins et al, 2014; Pellerito et al., 1992). Each of these conditions are a result of abnormal development or fusion of the Mullerian ducts and are commonly diagnosed using a HSG exam. Septate uterus is the most common abnormality in uterine development, affecting 0.2-2.3% of reproductive-age women (Rikken et al., 2020). The septum inside the uterus can be classified as partial or complete and is caused by improper fusion of the Mullerian ducts during development. Women with a septate uterus are at higher risk of recurrent pregnancy loss, preterm labor, and postpartum complications (Rikken et al., 2020). A surgical septum resection, or metroplasty can be used to surgically remove the septum but this procedure has not been linked to higher live birth rates (Rikken et al., 2020). A septate uterus can also be an improperly diagnosed bicornuate uterus, given their similar morphology. A bicornuate uterus is also a result of improper fusion of the Mullerian ducts, however this condition is described as a “heart-shaped” uterus and does not impact pregnancy outcomes as severely as the septate uterus condition (Kaur, 2021).

Unicornuate uterus is a condition characterized by development of a single uterine horn. In this instance, one Mullerian duct fully formed during development, but the second Mullerian duct degraded or halted development at a rudimentary state (Reichman et al, 2009). This condition often goes undiagnosed and does not pose a significant threat to fertility potential, but women with a unicornuate uterus may requiring more extensive monitoring during pregnancy due to increased likelihood of preterm labor (Reichman et al, 2009). Related to unicornuate uterus, uterine didelphys is a uterine malformation where each Mullerian duct develops independently and do not fuse at all during development (Heinonen, 1984). Patients with uterine didelphys have two uteri, each with one fallopian tube, ovary, cervix, and vaginal canal. Uterine didelphys can also go undiagnosed because it has little impact on pregnancy outcome, however, the condition can lead to painful/abnormal menstrual cycles and pain during intercourse. Correction of a didelphys uterus can be performed surgically if desired, but there is currently insufficient data on the effectiveness of this procedure for reproductive purposes (Rezai et al., 2015).

Endometriosis is one of the most prevalent conditions in women experiencing reduced fertility. According to a study conducted in 2010, roughly one-third of women with reduced fertility have some degree of endometriosis (Bulletti et al., 2010). Endometriosis is a heritable condition characterized by the development of uterine tissue outside of the body of the uterus—typically on or around the ovaries, fallopian tubes, or uterine ligaments, illustrated in Figure 9 (Matalliotaki et al., 2019). This condition can spread to other areas in the pelvic region as well but is less common. The exact link between endometriosis and infertility is unclear, but studies have shown that conditions

associated with endometriosis such as uterine adhesions and endometriotic cysts can negatively impact fertility potential (Arora & Mukhopadhaya, 2019). As with the harmful impact that pelvic adhesions can have on the fallopian tubes, uterine adhesions can bind up the uterus and cause uterine torsion, increasing the likelihood of miscarriage. Endometriotic cysts, called *endometriomas* release ROS and other inflammatory molecules that put stress on a developing embryo and induce deoxyribonucleic acid [DNA] damage (Sanchez et al., 2013). Removal of endometriotic tissue via laparoscopy is beneficial for maternal health and is highly recommended prior to pursuing fertility treatment (Tanbo & Fedorcsak, 2017). While many women with varying degrees of endometriosis have conceived naturally and produced healthy children, addressing endometriosis in patients struggling to conceive on their own increases the likelihood of successful treatment (Sun et al, 2020).

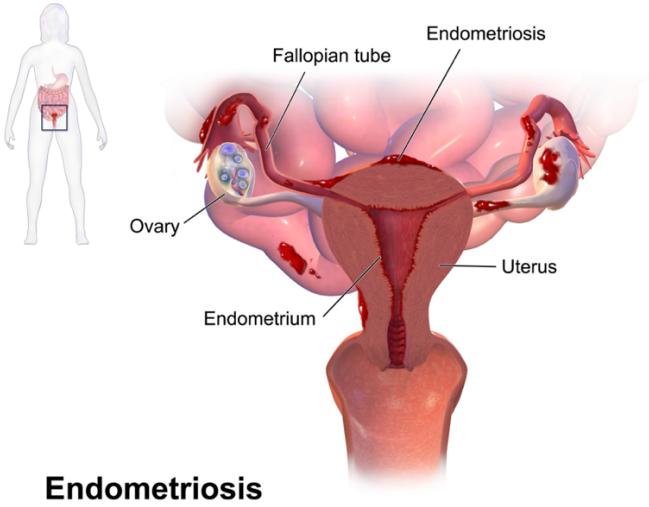

Figure 9. Endometriosis. From Blausen Endometriosis [Photograph], by BruceBlaus, 2014. Wikimedia Commons. (https://commons.wikimedia.org/wiki/File:Blausen_0349_Endometriosis.png). CC BY 3.0

Distinct from endometriosis, endometrial polyps are masses found in the uterine lining. While studies have not definitively concluded that presence of polyps leads to reduced fertility, there is speculation that uterine polyps may prevent proper embryo implantation into the uterine wall, leading to recurrent pregnancy loss (Taylor & Gomel, 2007). Most polyps are benign and cause few symptoms, if any at all (Sternberg et al., 2004). It is possible that polyps can naturally regress and disappear without treatment, but persistent uterine polyps may require removal and may still reoccur after regression or removal. Surgical removal of polyps is called a *polypectomy* and can be performed with or without anesthesia. Polypectomies are routine procedures performed in fertility clinics, especially for patients anticipating an embryo transfer from a previous IVF cycle. Despite the lack of conclusive studies on implantation rate and presence of polyps, some physicians opt to perform the procedure to give the patient the best possible chance for successful implantation (Ghaffari et al., 2016).

The final category of reduced fertility in women is cervical infertility. Cervical-factor infertility by means of stenosis or mucus hostility represent a small percentage of cases of diagnosed reduced fertility (Hull et al., 1985). Stenosis of the cervix can be caused by prior medical procedures, preexisting conditions or from genetic conditions. Reproductive cancers, recurrent dilation and curettage following miscarriages and cervical infections can cause narrowing or closing of the cervix, inhibiting the passage of sperm into the uterus. The cervix can be softened and dilated by an OBGYN or use of an ART can bypass the cervix for conception (Arora & Mishra, 2018). Cervical mucus hostility is a term that encompasses abnormal consistency or composition of cervical mucus. Cervical mucous that is too acidic, thick, dry, or contains anti-sperm antibodies

can cause sperm to die before they are able to pass through the cervix (Al-Daghistani, 2020). This can be caused by natural cycling of hormones during the menstrual cycle, medications, or the body's immune system. These forms of cervical-factor infertility are difficult to diagnose given the wide range of possible problems that can make the cervix and the mucous it produces less than ideal for conception. Typical treatment for cervical mucus hostility and cervical stenosis involves a standard ovulation induction protocol and artificial insemination (Felemban et al., 2018). The cervix can also have polyps that resemble uterine polyps which can prevent sperm from entering the uterus. Cervical polyps are most often benign and can be removed through a polypectomy and are less likely to reoccur thereafter (Budak & Kanmaz, 2019).

Human reproduction is a cooperative effort between both the male and female reproductive systems. Anatomy, physiology, and genetics influence sexual development and future reproductive potential of an embryo long before the reproductive system is fully formed. Throughout life there are some inevitable risk factors that impact fertility, as well as lifestyle choices that can undermine an individual's capacity to reproduce. These risk factors impact both men and women in different ways and play a much greater role in reproductive health than many patients realize.

Risk Factors Impacting Both Men and Women

Biological sex and health of the reproductive system play substantial roles in an individual's capacity to reproduce. There are additional risk factors unrelated to sex that can also impair fertility such as: age, diet, weight, tobacco/drug use, and alcohol consumption (Sharma et al., 2013). As discussed in Chapter 1, women are shifting traditional timeline of when they are starting families—lifestyle choices such as going to

college and entering the workforce have led to women becoming mothers later in life. The five-year increase seen in the age of first-time mothers from 1970 to 2017 does not typically correspond with a significant drop in fertility but does impact future family planning. Pregnancy rates have been shown to slightly decrease from ages 27-34 before dropping significantly from age 35-39 (Liu et al., 2011). Women are now starting their families at the upper threshold of their most fertile years, heading towards reduced fertility (Dunson et al., 2004). With the current age of first-time childbearing being approximately 26 years of age, if a woman decides to have a second or third child, her age will put her at risk for experiencing age-related reduced fertility with subsequent pregnancies (Guzzo & Payne, 2018). Whereas in the 1970's when women were starting their families in their early 20's, women could have two or three children before their age posed a risk to their fertility.

Women experiencing reduced fertility caused by other factors in conjunction with increasing maternal age may undergo earlier, more rapid loss of reproductive function manifesting as POI. Typically, women are diagnosed as sub- or infertile following one year of inability to conceive naturally—the standard definition of reduced fertility. However, after the age of 35, this window of time drops to just six months, making women over the age of 35 more likely to receive a poor prognosis on their fertility. As both the number and quality of oocytes decline, patients with advanced maternal age are more likely to have chromosomally abnormal embryos, and pregnancies more likely to end in miscarriage (Makhijani & Grow, 2020). Oocyte quality is traditionally assessed through visual parameters: appearance of the zona, ooplasm, polar body, and cumulus-oocyte complex morphology (Wang & Sun, 2007). Using visual methods to analyze

oocyte quality can roughly predict the likelihood of successful fertilization, but only in more extreme cases of obvious oocyte abnormalities. This method is not regarded as a reliable method of assessment; therefore, most clinics do not analyze oocytes for their quality, only for maturity(Wang & Sun, 2007). If maternal age has significantly compromised the ovarian reserve, or the quality of oocytes are too poor to fertilize and divide normally, use of an oocyte donor may be required to conceive (Makhijani & Grow, 2020).

Advanced paternal age also impacts male fertility, but to a far less severe extent. As with female fertility, overall fertility in men remains the same until their mid-30's. Studies have shown that semen quality remained relatively the same until around 34 years of age, but likelihood of experiencing erectile dysfunction did increase with age (Stone et al., 2013). Nearing age 40, overall semen quality began to decline--parameters such as sperm morphology, motility, concentration, and total semen volume declined each subsequent year (Stone et al., 2013). The observed decline in sperm quality associated with age is more likely to impact fertilization than pregnancy outcome. Sperm that are unable to swim properly due to morphological defects that impact their forward progression will be unable to pass through the cervix. If the ejaculate volume is low or contains very few sperm, it is less likely that the sperm will reach the unfertilized oocyte (Baskaran et al., 2020). In cases such as these, patients will often be recommended to pursue a method of fertility treatment such as IUI or IVF where sperm can be processed and utilized in its best condition. Donor sperm can also be used for patients with advanced age who have been unable to conceive—however, usage of donor sperm for cases of age-related infertility are less common than usage of donor oocytes for women.

In addition to a standard semen analysis, male patients over the age of 40 may also be asked to provide a semen sample for DNA fragmentation analysis—a parameter often associated with age (Ali & Parekh, 2020). A DNA fragmentation analysis reveals the percentage of broken DNA strands found in the sperm. Fragmentation of DNA in sperm is caused by an imbalance between reactive oxygen species levels and antioxidants found within the ejaculate fluid (Dorostghoal et al., 2017). Disturbance in this delicate balance leads to oxidative stress—the primary reason for strand breaks occurring in DNA. Short term oxidative stress can be a result of the immune system fighting an infection, but prolonged oxidative stress can cause toxic effects in the body and has been linked to the development of neurodegenerative diseases, cardiovascular disease, and age-related development of certain cancers (Patel & Chu. 2011). The results from a DNA fragmentation analysis alone cannot be used to diagnose a patient's reduced fertility, rather, it is used as a measure of fertility potential that can aide in narrowing down possible causes of reduced fertility. High percentages of DNA fragmentation in sperm can be a result of several different factors aside from advanced age, such as varicocele, chlamydia infection, poor diet, tobacco usage and drug abuse (Humm & Sakkas, 2013; Martínez-Soto et al., 2016; Sepaniak et al., 2006).

Increasing age is an inevitable part of life that cannot be changed, but most lifestyle choices are conscious decisions that can also impact fertility for men and women. Dietary choices and an individual's weight are often viewed as a cause and effect—poor diet leads to weight gain, and a healthy diet leads to weight loss or maintenance in healthy individuals. These factors are closely related, but an individual's weight is affected by much more than their diet alone. The relationship between diet and

fertility is a growing subject of study, but currently there are no specific clinical guidelines for patients trying to improve their fertility through dietary means. A healthy, varied diet that incorporates fresh fruits, vegetables, seafood, poultry, and nuts has been shown to improve semen quality in men, and antioxidant supplements are often recommended for men experiencing reduced fertility, especially in cases where advanced age is a factor (Nassan et al., 2018). Women are encouraged to pursue a similar diet, adding a folic acid supplement to their diet as well. Studies on the effect of folic acid during ovulation, conception and pregnancy have shown that embryo quality and conception rate improved with folic acid supplementation, and ovulatory dysfunction rates decreased (Chavarro et al., 2008). These recommendations are not only for men and women with reduced fertility—dietary patterns impact all organ systems in the human body and can lead to development of other medical conditions that will have an additive effect on a couple's inability to conceive naturally.

While dietary choices do contribute to an individual's weight, weight is regarded as a multi-faceted issue that can be tied to socioeconomic status, genetics, ethnicity, and other preexisting medical conditions as well. According to the National Center for Health Statistics, in 2017 over 42% of Americans classified as obese, and over the past 20 years, the severity of obesity has increased by nearly 5% (Hales et al., 2020). Obesity is a condition characterized by an abnormally high percentage of body fat—typically over 20% of the individual's normal body weight. Development of conditions as a result of obesity include type 2 diabetes, heart disease, high blood pressure, sleep disorders, and infertility. Fat tissue, also called adipose tissue, is a major site of energy storage and hormone production in the body. When present in excess, adipose tissue can over-

produce hormones that down-regulate reproductive function (Mitchell & Fantasia, 2016). In men, this can lead to oxidative stress and low levels of free testosterone in the body, which subsequently slows production of sperm and decreases the quality of the sperm that is produced (Katib, 2015). In women, obesity can also cause oxidative stress which impairs follicular development, leads to inflammation, and is also linked to the development or worsening of PCOS symptoms (Bhattacharya et al., 2010).

Use of legal and illegal drugs can have negative effects on both male and female fertility—including some medications advertised as fertility supplements that have not been approved by the Food and Drug Administration. Disclosing usage of drugs and supplements during a fertility treatment consultation can shape a patient's treatment plan and reveal potential sources of reduced fertility. Illicit substances such as methamphetamine, cocaine, and opioids can alter thyroid activity in the body which affects the menstrual cycle in women and sperm production and viability in men (Anderson et al., 2010). In addition to the negative impact illicit drugs have on conception, drug misuse is also commonly associated with more frequent miscarriages and fetal abnormalities (Arora & Tayade, 2018).

Depending on specific state legislature, cannabis may also be considered an illicit substance as well. Cannabis, or marijuana has a much more complicated and evolving legal history in the United States. At the federal level, cannabis is still classified as an illicit substance—although individual states have legalized cannabis for medicinal and recreational purposes. Marijuana use for medical purposes may help patients manage some of the symptoms associated with Human Immunodeficiency Virus [HIV] and Acquired Immunodeficiency Syndrome [AIDS], cancer, chronic migraines, epilepsy,

multiple sclerosis, Crohn's disease, and many other conditions that may or may not be treatable. While marijuana may alleviate some of these symptoms, studies have shown several unfavorable effects on the reproductive system (Ilnitsky & Van Uum, 2019). The principle psychoactive ingredient in cannabis, delta-9-tetrahydrocannabinol [THC], impacts production of LH by blocking the release of GnRH (Alvarez, 2015). In men this results in low testosterone levels and subsequent oligospermia, and women may experience irregular menstrual periods (Alvarez, 2015). Patients who disclose use of marijuana are typically recommended to abstain, if possible, for at least three months or more before pursuing fertility testing. By eliminating THC from the body for an extended period, the menstrual cycle may regulate itself normally and sperm production can increase naturally, thus eliminating the need for further fertility treatment (Payne et al., 2019). In cases where patients are managing symptoms from other conditions using marijuana or simply do not want to change their lifestyle, more invasive forms of fertility treatment are required but result in less likelihood of successful pregnancy outcomes overall (Klonoff-Cohen et al., 2006).

One of the most common forms of legal drug usage is tobacco, primarily in the form of smoking. Tobacco is one of the most extensively researched substances on human health and has been studied for decades. According to the CDC, prevalence of smoking in the United States has slowly declined from 20.9% in 2005 to 13.7% in 2018, but remains the leading cause of death, preventable diseases, and disabilities in the U.S. (CDC, 2019b). Both smoking and non-smoking methods of tobacco consumption have been shown to exhibit toxic effects on several major organs such as the lungs, skin, liver, heart, and stomach. Nicotine addiction, lung/oral/pancreatic cancer, chronic obstructive

pulmonary disease, and high blood pressure represent only a small fraction of the number of health complications that can arise as a consequence of tobacco use (Gallaway et al., 2018). One of the less commonly discussed repercussions of tobacco usage is the toxic effect posed on the reproductive system. Studies investigating the correlation between smoking and female fertility have shown a decrease in oocyte quality and accelerated depletion of the ovarian reserve, ultimately leading to premature menopause (Budani & Tiboni 2017; Hyland et al., 2015). Suboptimal oocyte quality can impair fertilization, lead to abnormal embryo development, and may be a physical manifestation of chromosomal abnormalities present in the embryo. Depletion of the ovarian reserve and poor oocyte quality are irreversible consequences which may render a patient incapable of having biological children—conception would then require use of an oocyte donor.

The male reproductive system is similarly impacted by tobacco usage. Findings from several observational studies comparing semen analysis parameters in smoking versus non-smoking patients found that use of tobacco products decreased viability, motility, and sperm concentration (Calogero et al., 2009; Harlev et al., 2015). Decreased motility has been linked to oxidative stress caused by spermatotoxic reagents in cigarette smoke, which negatively impact the volume and viscosity of seminal fluid (Harlev et al., 2015). The frequency of tobacco usage can also have a more deleterious impact on semen parameters. Patients who self-reported their tobacco usage as “heavy” often exhibited moderate to severe levels of teratozoospermia, asthenozoospermia, and oligozoospermia, whereas “light” smokers typically experienced only slight to moderate asthenozoospermia (Gaur et al., 2007). The adverse effects of smoking and non-smoking tobacco products on overall semen quality can be reduced over time and eventually

eliminated by quitting utilization of these products. Since the cycle of spermatogenesis is continual, over the course of several months all the defective sperm will either be ejaculated or reabsorbed by the body, and new healthy sperm can be produced again (Betts et al., 2013). Quitting tobacco consumption in any form is recommended not only for improving general quality of life, but preventing further damage to the body, including the reproductive system.

The relationship between alcohol consumption and reproductive health has long emphasized the teratogenic effects of alcohol on fetal development. The specific effects of alcohol on fertility in men and women have not been studied to the same extent, but studies have shown that routine alcohol consumption, especially in excess, can have detrimental effects on reproductive health in both sexes. Data from the 2018 National Survey on Drug Use and Health [NSDUH] showed that 86.3% of adults over the age of 18 have consumed alcohol at some point within their lifetime, and over half of those adults had drank within the last 30 days (NSDUH, 2019b). On its own, low to moderate consumption of alcohol has not been linked to significant reduction in fertility but is considered a contributing factor in reduced fertility (Gaskins & Chavarro, 2018). In cases of binge drinking and heavy alcohol consumption, alcoholism can impact hormonal regulation of estrogen in men which negatively impacts spermatogenesis (Grover et al., 2014). In addition to reducing sperm quantity in semen, alcoholism also affects the quality of sperm produced. In a 2017 study conducted on alcohol, smoking, and the additive effects that these factors have on fertility, men who routinely consumed alcohol had higher levels of DNA fragmentation and decondensation than the control group,

likely caused by heightened levels of reactive oxygen species in their semen (Aboulmaouahib et al., 2017).

Perspectives on the extent to which alcohol affects reproductive health in women has evolved and complexified significantly over the past 30 years. In the 1990's, several studies examined how alcohol impacted synthesis and utilization of estrogen, testosterone, LH and FSH in patients self-reporting regular alcohol consumption. Women who drank more frequently were more likely to exhibit higher levels of each hormone in their blood serum, which was reported to manipulate the menstrual cycle and even lead to anovulation (Muti et al., 1998; Reichman et al., 1993). These studies were later deemed inconclusive, as the reported values varied too greatly, and anovulation was rarely due to alcohol consumption alone. Due to the complexity of hormonal studies as well as the inconsistencies reported in them, researchers began to study alcohol in a different context: age. Studying the effect of alcohol on different age groups was presumed to yield more reliable results for two reasons: the relationship between age and fertility had already been established, and subjects could be classified in more clear-cut groups by their age rather than generalizing data from a large group of individuals based on fluctuating hormones.

In 2003, a Danish study examined prevalence of infertility in groups of women who consumed alcohol on a weekly basis. Their study concluded that alcohol did not affect female fertility in women below the age of 30 but was a significant predictor of infertility beyond age 30 (Tolstrup et al., 2003). This seemed to provide a more definitive answer than previous studies that reported their findings based on fluctuating hormone values. However, this study was quickly called into question when an 18-year analysis

conducted in Sweden between 1969-1987 was published the year after [2004]. Using hospitalization records, this study analyzed data from women between the ages of 18-28 and noted a strong trend between alcohol consumption and increased hospital visits for infertility and high-risk births (Eggert et al., 2004). These findings directly contradicted the Tolstrup et al. study published the year prior—the effect of alcohol was regarded as dose-dependent in the Eggert et al. study, whereas Tolstrup et al. stated that age determined the degree of toxicity that alcohol posed on the reproductive system. Following these publications, researchers have continued to make cases for both age-dependent and dose-dependent relationships between alcohol and infertility, but in more recent years, studies have advocated more for the dose-dependent relationship. Researchers have attempted to establish a quantitative amount of alcohol that is "safe" for consumption without affecting fertility. A Danish study published in 2016 observed menstrual cycle data on over 6,000 female patients between the ages of 21-45 and reported that less than 14 servings of alcohol per week had little to no impact on the time to conception (Mikkelsen et al., 2016). But the concept of a "serving" varies from country to country, and the amount of alcohol in a standard serving depends on the drink being consumed. Different varieties of beer, wine and spirits have varying alcohol contents. According to NIH's National Institute on Alcohol Abuse and Alcoholism [NIAAA], 14 grams of alcohol is the standard serving of alcohol in the U.S. (NIAAA, n.d.). By comparison, a standard drink in Denmark has only 12 grams of alcohol, and standard grams of alcohol present in drinks across Europe ranges from 8 grams up to 20 grams (Mongan & Long, 2015). As a result, discussing the relationship between alcohol and fertility on a global scale using servings of alcohol as a unit of measurement is not a

safe nor reliable method. Few studies have quantitatively analyzed alcohol and its effect on fertility using grams as the unit of measurement. Most recently, a meta-analysis published in 2017 reviewed data from over 98,000 women across eight countries to compare fertility across groups of nondrinkers, light, moderate and heave-drinkers (Fan et al., 2017). Through analysis, lower fertility rates were linked to consumption of alcohol that exceeded 12.5 grams per day (Fan et al., 2017). Currently, support for a dose-dependent relationship between alcohol and fertility outweighs those advocating for an age-dependent correlation. Decades of inconsistent and controversial publications on reproductive toxicity of alcohol still merit much further investigation. However, despite the lack of consensus, clinicians still recommend that women seeking fertility treatment abstain from drinking during treatment and pregnancy to mitigate any repercussions associated with alcohol consumption and reproductive health (ESHRE Task Force on Ethics and Law et al., 2010).

Reproductive anatomy, general/sexual health and lifestyle choices contribute substantially to fertility potential. Patients seeking fertility treatment are encouraged to disclose as much information as possible regarding their health to an OBGYN. Open discussions about all aspects of a patient's health can shape their treatment plan and potentially narrow down the source of reduced fertility. It is imperative that patients are made aware that there are factors outside of anatomy and physiology that may impact their fertility such as preexisting conditions, medications, and lifestyle choices. Adopting a healthier lifestyle benefits many areas of the human body, and the reproductive system is no exception.

Conclusion

The capacity of an individual to reproduce begins with proper formation of the reproductive system during early embryogenesis and continues into pubertal development. Improper development of the reproductive system can lead to reproductive dysfunction that may go unnoticed until later in life when an individual is struggling to conceive naturally. In this chapter I reviewed various diagnostic tests that are used in fertility clinics, including the clinic where I worked, to discern the source of reduced fertility in an individual. The blood serum immunoassay, standard semen analysis, hysterosalpingogram, and sonohysterogram are the most commonly performed methods of diagnostic fertility testing that provide insight into a patient's reproductive health. However, a patient's care does not end after they receive a diagnosis. Receiving a diagnosis helps the attending physician curate a personalized treatment plan for the patient that takes their specific needs and type/severity of reproductive dysfunction into consideration.

To conceive in the presence of reproductive dysfunction, fertility clinics provide treatment plans that utilize various forms of Assisted Reproductive Technologies, or ARTs, which can bypass certain forms of reduced fertility to allow a patient to conceive. Each form of reproductive technology will be described in Chapter 4: Fertility Treatments & Assisted Reproductive Technologies, including the average cost of treatment, commonly used medications, and the roles that laboratory staff and clinic staff play during the treatment process.

CHAPTER 4

FERTILITY TREATMENTS & ASSISTED REPRODUCTIVE TECHNOLOGIES

Synopsis

Fertility clinics specialize in providing medical assistance to patients who have been struggling to bear children naturally, typically using assisted reproductive technologies, or ARTs. Procedures such as ovulation induction, intrauterine insemination, and *in vitro* fertilization each aim to artificially bypass various forms of reduced fertility to help couples conceive. At the clinic where I worked, we provided fertility treatment services to patients from all walks of life—different ages, races, ethnicities, sexualities, socioeconomic statuses, family structures, etc. In this chapter, I discuss fertility treatments currently available for patients at the clinic where I conducted my clinical work, as well as those offered by most fertility clinics nationwide. I focused on the most common scenarios that I observed at the clinic: sub-fertile heterosexual couples, fertile homosexual couples, and infertile or sub-fertile single female patients. Starting with medications, I describe the various degrees of medicated treatment plans and how these medications play a role in the fertility treatment process. This will be followed by a description of the treatments themselves, in order of the least to most invasiveness treatment for the patient.

When describing each treatment, I wrote this chapter with patients in mind specifically—as a "what to expect" guide that outlines the various paths to parenthood for individuals interested in pursuing fertility treatment. As a member of the laboratory staff, my interactions with patients were not as extensive as the relationships fostered between patients and the nurses, medical assistants, and physician at the clinic. However, even in

my limited interactions, I noticed that many were interested in the process but didn't know how to ask about it. How did they receive a diagnosis of reduced fertility, but this clinic was able to work its magic and provide them with a child? This chapter answers precisely that question. Andrologists and embryologists at the clinic are often too busy to explain the intricacies of what goes on in the lab to every patient who is interested. In light of this, I wanted to help bridge the gap that exists between publicly available resources about fertility treatments for patients and the complex clinical terminology used amongst staff and in protocols at the clinic, to provide a resource that describes what goes on behind the scenes in the laboratory, where the so-called "magic" happens.

Beginning Treatment

Based on the results of the patient's fertility testing, the attending physician constructs a personalized fertility treatment plan for the patient(s) that takes into consideration the source(s) of infertility if applicable, the patient's treatment goals and their budget. The inevitable financial burden of receiving fertility treatment continues to be a limiting factor for the type of treatment patients choose to pursue. However, patients should always be informed of all their treatment options and be made aware of any methods that would not likely be successful for them. Severe forms of infertility may require more invasive forms of treatment, in which case less invasive methods are not likely to be successful. In some cases, a patient or couple may need to utilize additional services on top of the standard procedure to conceive, such as use of donor oocytes, donor sperm, or a gestational carrier. Fertility treatments vary in both cost and invasiveness, and there is a linear relationship between the two: the more invasive and involved a procedure is, the more costly the treatment becomes.

Regardless of the sexual orientation of the patient/couple, the overall procedure for each treatment is the same. Depending on the circumstances, what may change is the source of sperm or oocytes utilized for conception, or which individual will carry the pregnancy to term. Homosexual female couples and single female patients may not always be experiencing reduced fertility but still require a sperm donor to conceive, whereas homosexual male couples would minimally require both an oocyte donor and surrogate to have a child of their own. Heterosexual couples may be able to use their own sperm and oocytes, but in cases of severe infertility may be in a position where they require use of a sperm or oocyte donor, or perhaps a surrogate. Based on the initial consultation, the physician will determine if there is suspected infertility for one or both partners and order specific fertility tests to rule out reproductive disorders, and if needed, discuss options for using a sperm/oocyte donor.

Given the general limitations of insurance coverage for fertility treatments in the U.S., patients tend to prefer the lower cost options, although repeated failures of cheaper fertility treatments add up and eventually can cost more than if the patient had undergone one more expensive (but successful) cycle. Table 6: Breakdown of IUI/IVF costs provides an average price range for many aspects of IUI and IVF treatments. Success of any fertility treatment depends upon many factors, but in general, treatments that utilize ARTs to conceive tend to have higher success rates than those that do not. According to the American Pregnancy Association, the average success rate for an IUI cycle is 20% or less, compared to the average success rate of an IVF cycle that ranges from 23-43% for women who are less than 35 to 40 years of age (APA, 2017; APA, 2019). While both an IUI and IVF cycle are fertility treatments, there is an important distinction between a

fertility treatment versus an ART. A common misconception about ARTs is that any form of fertility treatment is considered an ART. This is because many people assume that a fertility treatment is a protocol or procedure that "assists" with reproduction. However, the way that the CDC and SART define what constitutes an ART is based on a U.S. Congressional Act passed in 1992 that states "The term 'assisted reproductive technology' means all treatments or procedures which include the handling of human oocytes or embryos, including *in vitro* fertilization, gamete intrafallopian transfer, zygote intrafallopian transfer, and such other specific technologies" (Fertility Clinic Success Rate and Certification Act, 1992). By this definition, fertility treatments such as timed intercourse, ovulation induction and IUI are not considered ARTs because no oocytes or embryos are handled—only sperm. Therefore, ARTs are a form of fertility treatment, but not all fertility treatments are classified as ARTs (CDC, 2021b).

Fertility Treatment Medications and Stimulated Cycles

With any fertility treatment plan comes the option to utilize fertility medications. They are not always required but may come as a strong recommendation from the physician depending on the patient's reproductive health status. The term "fertility medications" encompasses the numerous oral and injectable drugs used to control production and utilization of reproductive hormones within the body. Most fertility medications are taken by female patients and are self-administered, but there are several different medications that male patients can utilize as well. During a patient's consultation, they will receive information regarding the three different classifications of fertility treatment plans regarding medication usage: a natural cycle, *minimal stimulation* or mini-stim, or medicated ovulation induction [OI].

A “natural cycle” follows the patient’s menstrual cycle and will either require no medication at all or a short protocol of medications used at the end of their cycle to prevent premature ovulation (Allersma et al., 2013; Pelinck, 2002). This low or no-dose protocol is ideal for patients who ovulate normally on their own, those with high FSH levels, or patients that would like to reduce the overall cost of fertility treatment by not using medications (Schimberni et al., 2009). However, depending on the source and severity of reduced fertility, a natural cycle may not be successful for some patients—especially those who have a low chance of ovulating without use of a medication regimen (Von Wolff, 2019). Patients who may not be successful using the natural cycle protocol are those with irregular or anovulatory menstrual cycles, or patients with polycystic ovarian syndrome [PCOS], fallopian tube blockages, or suffering from primary ovarian insufficiency [POI] (Von Wolff, 2019).

If use of the natural cycle protocol is deemed insufficient for a patient, the next treatment plan option is a mini-stim cycle. The mini-stim protocol utilizes low, continual dosages of fertility medications throughout the entire cycle to regulate follicular development and ovulation (Zhang et al., 2016). Benefits of using the mini-stim IVF or other related treatment is a decreased cost of treatment, and fewer and less-severe side effects or decreased risk of the patient experiencing hyper-stimulation from the medications (Zhang et al., 2010). The proper dosage for each medication is determined by the attending nurses and physicians. Dosages can vary depending on the patient’s age, weight, reason for treatment and previous cycle history. Patients utilizing fertility medications of any kind are required to undergo continual monitoring of their cycle through both bloodwork and ultrasounds to assess how the patient is responding. Dosages

of each medication can be adjusted during the cycle depending on how well the patient is progressing under the current regimen. The mini-stim protocol tends to be a safe middle-ground for patients experiencing reduced fertility who do not want to undergo the traditional medication-intensive protocol. Through the mini-stim protocol, fewer follicles will grow and mature, limiting the risk of overstimulation and cycle cancellation, but does guarantee that the oocytes retrieved will be of good quality or have a high potential for fertilization (Zhang et al., 2016). The only protocol that will promote development of many follicles is medicated ovulation induction [OI].

Out of the three different treatment plans, medicated ovulation induction requires both the greatest number and highest dosage of fertility medications. Medicated OI is recommended for patients with anovulatory cycles or other severe forms of reduced fertility. Medicated OI is the most commonly utilized protocol for patients undergoing IVF, where it is considered optimal to retrieve as many oocytes as possible at one time. Increased use of fertility medications through the OI protocol stimulates a more significant number of follicles to undergo growth and maturation at a given time, and there are consequently more complications associated with this protocol than there are for a natural cycle or mini-stim protocol (Schimberni et al., 2009; Zhang et al., 2010). Complications and dangers of using self-administered injectable medications include pain, redness/swelling, infection at the injection site, accidental injection of air rather than the medication, and one of the most serious side effects, ovarian hyperstimulation syndrome [OHSS] (Rizk & Smitz, 1992). Due to continual blood and ultrasound monitoring of patients utilizing oral or injectable fertility medications for ovarian stimulation, cases of OHSS are often mild and treatable. However, if left untreated,

symptoms can become severe and in rare cases lead to patient fatality. Patients experiencing mild symptoms of OHSS may report nausea, fullness, and weight gain, but more severe complications can include vomiting, diarrhea, dark urine, shortness of breath, chest pain and abdominal distension (Gardner et al., 2001).

As with the mini-stim protocol, patients using the fully medicated ovulation induction protocol receive both verbal and written instructions about the medications they will be taking for their specific treatment plan and timeline. This includes information regarding proper medication storage, method of administration, timetables for medications and dosages for each medication that are calculated out for the patient. There is always potential for a patient to overuse OI stimulation medications which puts the patient at risk for developing OHSS, but this condition can also be a result of how the patient's body responds to certain medications. On average, incidence of OHSS in IVF patients is roughly 3-8%, but this value climbs to 10-20% for patients who have been diagnosed with PCOS (Farquhar et al., 2002). The exact link between PCOS and higher prevalence of OHSS is not fully understood, however, speculation suggests that Anti-Mullerian Hormone [AMH] levels before treatment indicate how the ovaries will respond to standard stimulation protocols. As a result, patients with high levels of AMH like those seen in patients with PCOS are more likely to experience ovarian hyperstimulation (Stracquadanio et al., 2017). Prevention of OHSS starts with the treatment plan: developing a customized, optimal stimulation protocol for each patient that uses the smallest amount of stimulation medications possible, but still induces sufficient follicular development and ovulation.

If OHSS is detected, the patient must stop taking all fertility medications which leads to cancellation of that cycle. This is done for the safety and wellbeing of the patient but is a devastating outcome of a treatment cycle. To the patient, a cancelled cycle is often viewed as either a failure by the fertility clinic treating them, by the patient's body, or both. A cancelled cycle will ultimately prolong a patient's journey to parenthood, but is a precautionary measure used to protect both the patient from serious health problems and the fertility clinic from medical malpractice.

However, OHSS is not the only reason for cycle cancellation. The need to cancel a patient's cycle can be a result of poor ovarian response (leading to too few follicles), or excessive response leading to too many follicles for patients who are pursuing IUI or TIC where there would be a high risk for multiple gestation (Kailasam, 2004). In the event that a patient's cycle needs to be cancelled, this information must be included in the cycle data reported annually to the CDC and SART. Most physicians will use previous cycle data to formulate new stimulation protocols for unsuccessful IVF patients if the patient decides to undergo treatment again. Cycle cancellations are not ideal for the clinic or patient, so proper dosage calculations/adjustments and meticulous monitoring help ensure the patient can avoid cancellation and complete their cycle.

Oral and injectable medications routinely used for cycle regulation and ovulation induction are outlined in Table 7: Common Medications Used in Fertility Medicine. Medications are broken down into four different categories based on the stage of the patient's cycle at which they are used: Suppression, Stimulation, Trigger, and Other: Post-OI. Within each category, different potential medications can be administered, with possibly more than one medication from a particular category. For example, during the

stimulation stage, one patient may use a combination of both injectable FSH and LH medications, while another patient may only use injectable Human Menopausal Gonadotropin. Variations in the medication protocols can be a matter of the physician's/patient's preference or decided upon based on the patient's history with fertility medications. Determining the appropriate dosage of each medication is dependent upon numerous factors such as age, weight, type and concentration of medication, and any other medications being taken that may interfere with the effectiveness of fertility treatments. Nurses and physicians determine what dosages the patient will administer for each medication. Specific dosages for each patient reflect the high level of variability and number of medications prescribed, type, and dosage used as well as differences in response variability from patient to patient. In Table 7, example medication protocols for each treatment plan are listed in their corresponding subheadings.

Timed Intercourse

Timed Intercourse [TIC] is the least invasive and most affordable fertility treatment option for patients and tends to be more effective for patients with no known source of reduced fertility or for women with irregular/absent menstrual cycles. This simple treatment plan utilizes the patient's natural menstrual cycle to optimize sexual intercourse and increase the likelihood of conception (Agarwal & Haney, 1994). At the clinic where I worked, we did not implement a TIC protocol very often, as most of our patients needed more involved forms of treatment. Within a regular menstrual cycle, ovulation occurs around day 14—with a 6 day "fertile window" where conception is most likely, which begins four or five days before ovulation and ends on the day ovulation

occurs (Manders et al., 2015). Women with consistent menstrual cycles can more easily predict their fertile window without testing, but for those with inconsistent menstrual cycles, patients may need to use a combination of cycle tracking, basal body temperature tracking and ovulation test kits to determine when ovulation will occur (Manders et al., 2015).

The basal body temperature method uses daily temperature readings to determine when ovulation has occurred. Correlations have been made between a woman's basal body temperature and the time ovulation, typically characterized by an increase in body temperature by 1ºF or less (Su et al., 2017). The rise in more reliable and convenient ovulation test kits available at drug stores and local pharmacies in the U.S. has made the basal body temperature method less frequently used. Ovulation test kits function similarly to urine pregnancy tests—both are rapid, qualitative tests that measure the presence of specific molecules in urine. Pregnancy test strips analyze for the presence of hCG, while ovulation test kits test for the surge in LH that occurs post-ovulation (Su et al., 2017).

Over the past decade, keeping track of the many details of a patient's menstrual cycle has become a largely digitized process. Menstrual cycle calendar applications, commonly called "Period Tracker Apps" are available on nearly all smartphones in use today. Within these applications, patients can chart their menstrual cycle data: when their period begins and ends, the amount of discharge, basal body temperature, changes in cervical mucus, fluctuations in mood and other cycle symptoms. Some also have algorithms that compile cycle information and can help to predict the patient's fertile window based on previous months of cycle tracking. The convenience and individualized

nature of these applications makes TIC a treatment plan that requires little if any medical intervention.

One benefit of the TIC treatment plan is that it follows the patient's natural cycle and does not require medications—this saves the patient both money and time since there are no trips to the fertility clinic for monitoring, nor trips to the pharmacy to pick up medications. An unmedicated TIC cycle is typically called "Natural Family Planning" since the entire cycle relies solely on the patient's natural menstrual cycle. While Natural Family Planning may work for some patients, a completely unmedicated cycle may not be sufficient for patients to conceive. Patients who are struggling to become pregnant using Natural Family Planning, especially those with reduced fertility related to PCOS, may be more successful using fertility medications for ovulation induction alongside timed intercourse (Abu Hashim et al., 2011). Clomiphene Citrate, or Clomid is the most common medication recommended for TIC patients, as mild doses of Clomid can encourage just one or two oocytes to mature at a time as opposed to dozens in a fully medicated cycle (Progyny, 2020). Some facilities may offer patients the option to use injectable medications for TIC, but in most cases, if injectable medications are needed physicians will encourage patients to pursue intrauterine insemination to maximize their cycle further.

Clomid is taken orally for a period of five days, starting three days after a woman's menstrual period begins and works by stimulating production of FSH and LH from the pituitary gland by inhibiting production of estrogen (Advanced Fertility Center of Chicago, 2020a). Higher levels of FSH and LH in the body induce production and growth of dominant follicles in the ovary, and the size of these follicles are monitored via

transvaginal ultrasound. Patients who are unresponsive to Clomid may take Letrozole instead (often sold under the brand name Femara), which is an orally administered aromatase inhibitor that works similarly to Clomid by inhibiting production of estrogen to encourage the pituitary to produce more FSH and LH (Kar, 2013). Femara tends to be the preferred medication used for ovulation induction in patients with polycystic ovarian syndrome but can also be used for patients who do not respond well to Clomid (Legro et al., 2014). Even though Clomid and Femara are taken only for a brief period and are not given in high doses, any use of stimulation medications requires monitoring through transvaginal ultrasound and/or bloodwork. The clinic where I worked scheduled blood draws every other day, or every three days at most when one of our patients was taking oral or injectable stimulation medications. Stimulation of the ovaries can lead to too many follicles developing at once or put the patient at risk for OHSS if hyperstimulation goes undetected. Ultimately the use of medications for ovulation induction come with increased cost, time commitment and physical risk but may increase the likelihood of conception for the patient.

Regardless of whether medications are used, properly timing sexual intercourse around ovulation is crucial for optimizing the possibility of successful conception (Manders et al., 2015). A common misconception surrounding frequency of intercourse and conception is that "more is better." Couples having intercourse every day, even within the fertile window are not actually more likely to conceive. While the cycle of spermatogenesis is rapid enough to replenish sperm daily, patients are recommended to have between 2-7 days of abstinence before having sexual intercourse to optimize semen parameters according to the WHO (WHO, 2010). Since sperm can also survive in the

fallopian tube for several days before fertilization occurs, having intercourse every day will not significantly improve chances of conception (Suarez & Pacey, 2005). Two weeks following ovulation, a urine pregnancy test can confirm conception—testing prematurely may yield a false negative because the patient's hCG level is too low to be detected through urine (APA, 2020). Quantitative blood serum assays can detect much smaller amounts of hCG and can confirm a pregnancy several days earlier than a urine test (APA, 2020).

Intrauterine Insemination

Many protocols utilized in fertility medicine today were originally developed for use in the agricultural industry. Artificial Insemination [AINSEM] is one of the earliest techniques used in fertility clinics that was initially popularized by its early success in agricultural animals. AINSEM is a process by which semen is loaded into a long catheter, fed through the cervix into the uterus and directly placed in the uterine cavity. This process was first performed in animals successfully in the 1700's and has been used for centuries since then as a more efficient and safe means for breeding animals (Ombelet & Robays, 2015). Results of artificial insemination were not always efficient and reliable, however. An early lack of knowledge regarding both human and animal reproduction in the 18th and 19th centuries meant many failed AINSEM's. Over time, AINSEM protocols improved with more knowledge from human and animal studies. In 1920, Russian biologist Ilya Ivanoff published a protocol that most closely resembles the modern AINSEM protocol utilized in fertility clinics today, and he coined the term Intrauterine Insemination, or "IUI" (Ombelet & Robays, 2015). In humans, IUI may be beneficial or even necessary for someone to conceive. Female patients with anovulatory or irregular

cycles, endometriosis, or cervical factor infertility may benefit from IUI, as well as couples struggling to conceive due to mild male-factor infertility—though it is not a replacement for a more invasive procedure like IVF (ESHRE Capri Workshop Group, 2009). The IUI procedure bypasses two of the largest barriers the sperm must overcome: the vaginal canal and cervix. By placing sperm directly into the uterine cavity, a greater number of sperm have a chance to fertilize the oocyte, as the distance that the sperm must travel to reach fallopian tube is decreased.

As with TIC, an IUI can follow a patient's natural menstrual cycle and use no form of fertility medications, with what is called a "natural cycle," or can use medications to induce ovulation. Medicated IUI cycles are more common than natural IUI's, despite the increased cost and risk associated with using fertility medications. Patients undergoing controlled ovarian stimulation for their IUI begin their stimulation protocol or "stim" on day 3 of their menstrual cycle (Barad, 2018). The average duration of an IUI stim is 8 days but can range from 5-14 days depending on how the patient responds to the medications. Most clinics perform a baseline transvaginal ultrasound on day 3 to observe the state of the uterus, fallopian tubes, and ovaries, with blood work to establish a hormonal baseline for the patient. If the tests do not reveal any abnormalities or reasons for concern, the patient is approved to begin ovarian stimulation. The patient is given instructions on the timing and dosage of medications they will be using for their cycle. Numerous different combinations of medications can be used to stimulate follicle development and ovulation for an IUI, but the most common combinations are: Clomid or Femara alone, Clomid/Femara with an hCG trigger shot, Follistim/hCG, hMG/hCG (Barad, 2018). All three methods of controlled ovarian stimulation have been proven

effective and the physician will typically decide the best stim protocol for each patient based on age, cycle history and reason for reduced fertility.

Stim medications are taken/administered daily, and routine bloodwork and ultrasound monitoring are conducted to ensure proper ovarian response, monitor for OHSS, and ensure no cysts are forming from the stim medications. Timelines are at the discretion of the clinic, but at the clinic where I worked, blood draws to monitor levels of estrogen and FSH were conducted every other day until the patient's IUI appointment. At the clinic, ultrasound monitoring of follicular number and size began around day 7-8 of the patient's cycle—about 4-5 days after starting the stimulation protocol. Follicle number is especially important for IUI cycles as there is a significant risk of multiple gestation if too many follicles are stimulated at once. For a typical IUI cycle, only one or two follicles should be growing at once (Practice Committee of the American Society for Reproductive Medicine, 2012). The likelihood of the patient's cycle being cancelled increases when three or more follicles are developing at the same time. Once the dominant follicle(s) reaches between 19-20mm in diameter, the patient is instructed to self-administer a Human Chorionic Gonadotropin (hCG) trigger shot to trigger ovulation and the patient is asked to return to the clinic 36 hours later for her IUI procedure (Maher et al., 2017).

If the patient is using a fresh semen sample for the procedure (either from the patient's partner, or from a donor), the sample can be collected at the fertility clinic, or at home as long as the fertility clinic laboratory receives it no later than one hour after collection. The laboratory staff analyzes the raw sample to observe its initial quality before processing it to optimize the sample for the IUI procedure. Semen samples IUI are

typically processed in one of three ways: a simple wash, the swim-up method, or with density gradient centrifugation. The simple sperm wash and sperm swim-up methods are more common for n9ormal semen samples, whereas density gradient centrifugation is optimal for samples with low concentration, abnormal sperm, or high amounts of debris (WHO, 2010). Washing sperm is the simplest procedure which involves diluting semen in sperm wash media, centrifugation, and discarding the supernatant--the liquid that sits above the pellet of sperm after centrifugation. The swim-up method is a bit more involved—semen may be washed before performing a swim-up, depending on the initial quality of the semen initially. The sample is then loaded into a test tube and briefly centrifuged to form a loose pellet of sperm at the bottom of the tube (Jameel, 2008). Then, clean, pre-warmed culture medium is floated on top of the sperm.

This hospitable, nutrient-rich medium encourages the motile, healthy sperm to swim up into it, leaving the nonmotile sperm and debris at the bottom of the tube. The tube is incubated for an hour, giving the sperm time to swim up into the medium before the medium is collected and washed again prior to use for the IUI (Jameel, 2008). The last protocol is density gradient centrifugation. At the clinic, this is the method of sperm prep we utilized most often. Out protocol for density gradient centrifugation used either a 45% gradient, or a combination of 45% and 90% gradient. The gradient is composed of microscopic silica beads (either 45% of the solution, or 90% of the solution) that are suspended in a HEPES-buffered media (Shivani Scientific, 2014). When preparing semen for an IUI, we would typically process semen on the lower-density 45% gradient only. Depending on the quality of the sample, however, if extra debris needed to be removed, we would run the sample on a combination of both 45% and 90% gradient to yield a

better sample for the patient. A single or combination gradient helps removes debris, white blood cells, and nonmotile and abnormal sperm that are present in the raw sample, leaving only the motile, good-quality sperm behind.

To load the gradient, we would prepare a clean 15mL conical falcon tube labeled with all pertinent patient information and pipette 1mL of the 45% gradient is into the bottom of the empty tube. Then, if needed, we carefully pipetted 1mL of 90% gradient underneath the 45% gradient so two distinct layers form with the 45% on top and the 90% at the bottom. If the layers mix, or the gradient will not be effective. From that point, our protocol allowed up to 4mL of raw semen to be floated on top of the gradient. In the event that we had >4ml of raw semen, we prepared a second gradient using the same method, and the remaining semen was placed on the second gradient. Floating more than 4mL of semen on a single gradient prevents all the sperm from being pulled through the gradient properly. Density gradient centrifugation works by separating out cells in a liquid sample based on their size and mass (Coumans et al., 2017). After centrifugation, debris and broken sperm are caught in the uppermost portion of the gradient and can be discarded—leaving the healthy, motile sperm at the bottom of the tube. The denser the gradient, the fewer sperm will be able to make it through the gradient. However, semen run on a denser gradient will typically yield fewer but higher quality sperm than semen run through a less dense gradient—although this can vary greatly depending on the initial quality of the raw semen sample. If we needed to use two gradients due to a high-volume sample, the resulting pellets at the bottom of each tube are combined into one tube, then the sample is washed one final time with pre-warmed sperm wash medium before being placed in the incubator awaiting the patient's IUI procedure.

While using fresh semen is best, some patients had IUI's using frozen vials of sperm that were frozen weeks, months or even years before the procedure. Frozen semen can come from previous collections from the patient's partner or using sperm from a known or anonymous sperm donor. Semen can be frozen in its raw, unprocessed form which requires processing after thawing. If the patient knows they will be pursuing an IUI, they can have sperm frozen for them pre-processed as IUI-ready vials (or purchased from a donor as an IUI-ready vial) that can be thawed, washed, and used without performing any additional processing. Vials frozen as IUI-ready are not necessarily advantageous in any way for the patient, they are just pre-processed and require less prep-time for the laboratory staff on the day of the IUI.

When the patient arrives for her IUI, she is taken into a private exam room for the procedure. At our clinic, both the physician and nurse practitioner would perform IUI procedures, accompanied by a medical assistant who set up the room beforehand. The patient lies on the exam table with her feet placed in the attached stirrups. A clean speculum inserted into the patient's vaginal canal by the physician/nurse practitioner, opens the vagina for the IUI procedure. The patient is asked to confirm her name and date of birth, which is verified with what is written on the tube of sperm processed by the laboratory staff to make sure the correct sample is being used for the patient. The processed sperm is drawn into a long, flexible sterile catheter using a syringe. The tube of the catheter is then fed through the patient's cervix and the sperm sample is expelled directly into the uterine cavity (Figure 10). After the sperm is inside the uterus, the catheter can be removed, followed by the speculum and the IUI procedure is complete. Overall, our lab required 1-2 hours of lead time to prepare a semen sample for an IUI

procedure, but the IUI procedure itself takes 10-15 minutes. No earlier than two weeks following the IUI procedure, the patient can determine if the IUI was successful through an at-home urine-based pregnancy test, or through a blood draw at the clinic to measure hCG levels.

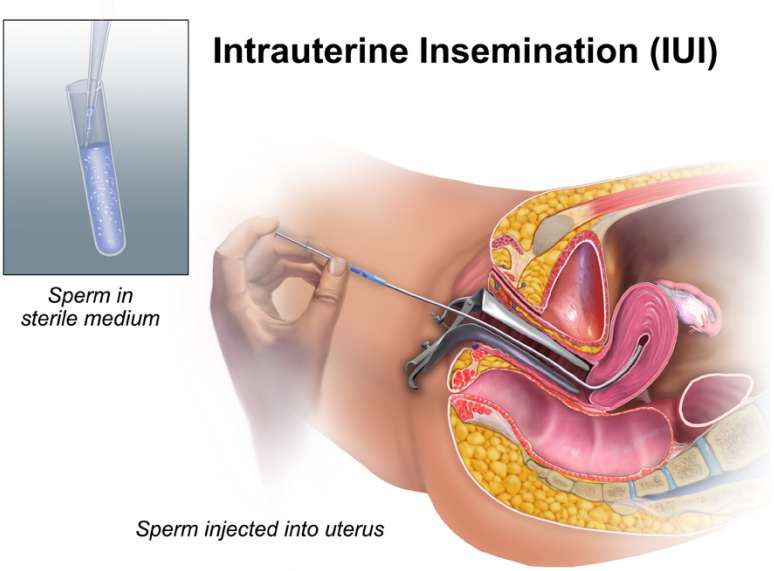

Figure 10. Intrauterine Insemination (IUI). From IUI [Photograph], by BruceBlaus, 2017, Wikimedia Commons (https://commons.wikimedia.org/wiki/File:IUI.png). CC BY-SA 4.0

In Vitro **Fertilization**

In Vitro Fertilization, or IVF is the most complex and costly fertility treatment currently available for patients. The phrase "*in vitro*" is a Latin phrase that means "in glass"—meaning IVF is a procedure by which oocytes are physically removed from the body and fertilized inside of a sterile dish in the laboratory (Zhu, 2009). This treatment may be utilized if couples have experienced repeated IUI cycle failures, recurrent

pregnancy loss following an IUI, if their infertility is severe enough that IUI's are not a viable option of treatment, or in more recent years as a means for gender-selection. IVF was first successfully performed in the late 1970's when Patrick Steptoe and Robert Edwards, respectively a gynecologist and professor of human reproduction from the United Kingdom worked together to create embryos in a laboratory for infertile couple Lesley and John Brown (Hartshorne, 2008). After successful fertilization of Lesley's oocytes using John's sperm, Steptoe and Edwards transferred an embryo into Lesley who gave birth to Louise Brown on July 25th, 1978. Since then, millions of babies have been born using IVF across the globe.

The goal of an IVF cycle is to retrieve as many oocytes as possible. Since the oocytes are fertilized in the lab and only one or two embryos are transferred back into the uterus at a time, having numerous follicles growing at once does not pose a risk for multiple gestation like it would during an IUI cycle. Patients have ultimate control over whether they want one or two embryos transferred, and any excess embryos allow the patient to undergo additional later transfers if their first was unsuccessful. Most IVF patients follow the conventional ovarian stimulation protocol for IVF which uses several injectable medications throughout the cycle to stimulate as many follicles as possible—but there are different treatment plans for different patient's needs.

Natural IVF is exactly like natural family planning or a natural IUI cycle—treatment follows the patient's menstrual cycle, and no medications are used. Abstaining from using any fertility medications decreases the likelihood of developing OHSS and cuts down the cost of receiving IVF treatment. However, since only one follicle grows to maturity and is ovulated during a typical menstrual cycle, that means that only one oocyte

can be potentially retrieved and fertilized in the lab, significantly decreasing the likelihood of having a healthy embryo to transfer into the uterus later on. Most clinics do not perform natural cycle IVF due to its extremely low success rates and encourage the "mini-stim" protocol instead. A mini-stim cycle uses more medications than a natural cycle, but fewer (and in smaller doses) than conventional ovarian stimulation for IVF (Progyny, 2020). The mini-stim protocol either involves oral medications to stimulate and a single trigger shot injection similar to a medicated IUI cycle or utilizes a combination of oral and injectable medications: Clomid or Femara for the first 5 days and an FSH injection for the last 10 days before triggering ovulation (Progyny, 2020). Regardless of the mini-stim protocol used, a mini-stim cycle is still cheaper than conventional IVF and requires fewer daily injections. Although similar to a natural cycle, using few (if any) medications will not yield the same number of oocytes that would be expected for a patient that chooses to undergo the full IVF stimulation protocol (Progyny, 2020).

Conventional IVF is costly, requires numerous daily injections and does not typically use any oral medications other than oral contraceptive pills at the start of their cycle. There are three protocols used for conventional IVF: the long Lupron protocol, antagonist protocol and microdose Lupron flare protocol (Advanced Fertility Center of Chicago, 2020b). The Long Lupron protocol, also called Luteal Lupron protocol is the longest protocol that takes roughly 42-44 days from the start of contraceptive downregulation to the retrieval date—with some variation from patient to patient. Following the Luteal Lupron protocol, upon the start of the patient's menstrual cycle, they are instructed to come into the clinic for a baseline blood draw on day 3 of their

cycle and will begin to take a daily oral contraceptive pill [OCP] for up to 21 days (USC Fertility, 2019; Coastal Fertility, 2021). Taking an OCP seems counterintuitive to many patients who are seeking fertility treatment to do the exact opposite of the goal of birth control. However, OCPs serve several important purposes in the Luteal Lupron protocol: for clinic scheduling (so only a few patients are undergoing oocyte-retrievals in a single day), to prevent cysts from forming later in the patient's cycle, and to begin the process of downregulation (USC Fertility, 2019). Downregulation allows for better control over the patient's cycle, inhibits premature ovulation and ensures that all follicles in the ovary will start off at the same size for the stimulation phase of treatment. With all follicles in the ovary at the same size before starting stimulation, each follicle will be stimulated at roughly the same rate so all active follicles should ovulate mature oocytes during the trigger and can be retrieved at the same time.

Lupron is the most commonly used downregulation medication that effectively turns off production of reproductive hormones in the pituitary gland so the LH surge that occurs during ovulation cannot occur (Advanced Fertility Center of Chicago, 2020c; Coastal Fertility, 2021). On day 15 of taking an OCP, the patient begins self-administering a daily Lupron injection as well (Coastal Fertility, 2021). The patient will continue both medications until their OCP runs out on day 21 and then administer only Lupron for another 5-10 days until their menstrual period begins—this specific timing of the Lupron start is clinically referred to as "mid-luteal" timing. A blood draw is scheduled to assess estrogen levels following the start of the patient's menses, and after the baseline is established, the patient administers one more Lupron-only injection before stimulation begins. The following day is "Stim Day 1"—the patient's daily Lupron dose

is cut in half and the patient begins taking an injectable form of FSH such as Follistim or Gonal-F, or an hMG injectable such as Menopur (Coastal Fertility, 2021). Daily low-dose Lupron and either FSH or hMG injections continue until stim day 10, with bloodwork and ultrasounds conducted every other day to monitor follicle growth (USC Fertility, 2019). When the majority of the follicles present in the ovary fall between 18-22mm in size, the patient is instructed to administer their trigger shot of hCG and return to the clinic 36 hours later for their oocyte retrieval (Advanced Fertility Center of Chicago, 2020c; Coastal Fertility, 2021). If the follicles are not large enough yet, the patient will continue their low-dose Lupron and FSH/hMG injectables until instructed to stop and can administer their trigger injection (USC Fertility, 2019). Given the extended period of time that patients are taking injectable fertility medications, this requires extreme flexibility with the patient's schedule and are at much higher risk for "over-suppression" by the Lupron protocol which may cause the patient to respond poorly to stimulation medications and few follicles will mature.

To reduce the likelihood of over-suppression and use fewer injections, the antagonist protocol was developed as a slightly shorter protocol for conventional IVF. Similar to the Luteal Lupron protocol, upon the start of the patient's menstrual cycle, the patient has a baseline blood draw performed on day 3 of their menstrual cycle and begins to take a daily OCP. In the antagonist protocol, the patient takes an OCP for only 17 days, then stops taking the OCP for four days before starting their stim protocol (San Diego Fertility Center, 2006). On stim day one, the patient begins to self-administer a daily injection of FSH and have bloodwork and ultrasound monitoring every other day until the date of her retrieval, depending on the physician's preference (Progyny, 2020). Starting

on stim day six, the patient must also begin a daily Ganirelix or Cetrotide injection as well. Ganirelix and Cetrotide are GnRH antagonists that block GnRH binding sites in the pituitary which prevents the LH surge during ovulation (Advanced Fertility Center of Chicago, 2020d). Daily injections of FSH and Ganirelix/Cetrotide continue until the follicles reach maturity and the trigger shot can be administered (Advanced Fertility Center of Chicago, 2020d). An hCG trigger shot can be utilized to trigger ovulation but is not recommended for the antagonist protocol by itself—a Lupron injection is more often used as a trigger for the antagonist protocol as it causes a sufficient surge in FSH and LH to trigger ovulation without inducing hyperstimulation and can also be administered as a dual trigger shot as a combination of both Lupron and hCG to ensure that ovulation occurs (Lin et al., 2019). Following the trigger shot, the patient will return to the clinic for her scheduled oocyte retrieval. The antagonist protocol takes roughly 32-34 days from the start of OCPs to oocyte retrieval and is significantly shorter than the Luteal Lupron protocol (Advanced Fertility Center of Chicago, 2020e).

The shortest stim protocol for IVF is the microdose Lupron flare protocol. This protocol provides a jump start to ovarian stimulation and may be the preferred protocol for women with increased maternal age or who are poor responders to longer protocols (Progyny, 2020). As always, this protocol begins with OCPs—14 days of OCP's starting on day 3 of the patient's menstrual cycle. After completing two weeks of daily OCP's, the patient stops their contraceptive for two days before starting their brief downregulation period (Advanced Fertility Center of Chicago, 2020f). The second day without OCP's marks the start of bloodwork and ultrasound monitoring every other day. On day three without contraceptives, the patient begins to self-administer a diluted

Lupron dose twice a day—half of the normal dose in the morning and the other half at night (Advanced Fertility Center of Chicago, 2020f). By taking half of the dose twice a day, the patient is still administering a full dose of Lupron each day but provides a more continual dose throughout the day. Twice-daily injections of diluted Lupron continue for a second day and on day three the patient begins their daily stim injection of FSH (Advanced Fertility Center of Chicago, 2020f). A typical stim for the microdose Lupron flare lasts 8-11 days and the patient will continue their dilute Lupron and FSH injections until the follicles in the ovary reach maturity. At that point the patient is instructed to stop administering Lupron and FSH and administer their hCG trigger shot that evening and return to the clinic 36 hours later for their oocyte retrieval, for a total time of 25-27 days between the start of OCPs to oocyte retrieval (Progyny, 2020).

The day of the oocyte retrieval, the patient arrives at the clinic and prepares for the retrieval procedure. Transvaginal oocyte retrievals are performed by the OGBYN, accompanied by an anesthesiologist that has given the patient an intravenously administered anesthetic—Monitored Anesthesia Care [MAC] (Nagarajan & Lew, 2018). MAC is a form of localized anesthetic that is combined with sedation and analgesia (Kwan et al., 2013). Patients receiving this propofol-based anesthetic are aware but relaxed and do not need respiratory support during their procedure (Nagarajan & Lew, 2018). This allows for a faster post-operative recovery time and there are fewer side effects associated with MAC versus traditional general anesthesia—patients are instructed to not eat or drink the night before or morning of their retrieval procedure (Kwan et al., 2013).

To begin the oocyte retrieval process, the physician cleans the vaginal opening and cervix. A transvaginal ultrasound probe is used to visualize the ovarian follicles and serves as a guide for the physician throughout the retrieval (CNY Fertility, 2020b). The probe is fitted with a long sterile needle for the procedure that is connected to a sterile test tube filled with warm media for oocyte collection. The transvaginal probe is inserted into the vagina and the follicles come into view on a screen inside of the procedure room (Mayo Clinic, 2019e). Using the view of the follicles as a guide, the physician moves the needle over to a follicle, punctures the wall of the ovary with the needle tip and aspirates the follicular fluid until the follicle walls visibly collapse on the screen (CNY Fertility, 2020b). Individual oocytes are not visible on the screen, but once the follicle borders collapse, the fluid inside of the follicle is aspirated and the oocyte will be floating in the fluid in the collection tube. Once the tube is nearly full, it is capped and passed through into the lab where an embryologist will empty the tube into a dish and begin collecting oocytes out of the follicular fluid (CNY Fertility, 2020b). In doing this, the embryologist can count the exact number of oocytes retrieved and separate the oocytes out of the fluid that may contain blood clots or torn tissue which may stick to the oocytes over time and make them difficult to see.

Removing the oocytes from the follicular fluid and placing them in a more pH-stable media is also important to prevent contamination and any cytotoxic changes in the osmolarity of the oocytes (Mayo Clinic, 2019e). The process of follicular aspiration and oocyte counting continues until the final mature follicle of the patient's ovary has been punctured, aspirated, and collapsed. Following completion of the retrieval, the intravenous sedative is removed, and the patient is monitored by the anesthesiologist until

she regains consciousness (CNY Fertility, 2020b). Attending nurses move the patient to a recovery room so they can relax and continue to regain their strength for a few hours until they leave the clinic. Patients undergoing a fresh embryo transfer will begin to take progesterone suppositories the evening following their retrieval to prepare their uterine lining for transfer. Fresh embryo transfers are not common practice today and were seldom performed at the clinic where I worked—the overall process of downregulation, stimulation and oocyte retrieval is very taxing on the body, so many physicians prefer to freeze embryos for at least one cycle so the patient's body can recover fully before an embryo transfer.

Back in the laboratory, embryologists finalize the number of oocytes collected during the patient's retrieval procedure and report the final count to the physician. At the clinic where I worked, during "retrieval week", a new patient was in the procedure room for a retrieval every 30 minutes—so the turnaround was very quick. After the retrieval is complete and the oocytes had been counted and reported, embryologists in our lab would close each patient's dish of oocytes, then place them into a warm, humidified chamber for 3-6 hours so the oocytes could continue to mature and equilibrate to the surrounding culture media until all the retrievals for the day were over. During that window of time, the lab reviewed the patient's signed consent forms and orders. "Orders" referred to documents signed by the patient (and their partner, if applicable) that state what the patient would like performed on their oocytes/embryos, who the sperm source will be if the oocytes are to be fertilized in the lab, and what will be done with extra embryos (frozen for future use, ethical discard, donation, etc.). The clinic required signed and notarized permissions to be scanned into the patient's file before any procedure could be

performed using their oocytes. and must be completed prior to the patient's retrieval, as orders cannot be signed after the patient has undergone anesthesia.

Based upon the patient's signed orders, the retrieved oocytes can be used in several ways: oocytes could be frozen for the patient to use later on (called "autologous oocyte banking"), used fresh or frozen for another patient's use as donor oocytes, or could be inseminated the day of the retrieval to create embryos for the patient. The intended use of the oocytes determines which protocols to use to fertilize and/or preserve them. Conventional insemination [INSEM] was one of the most common protocols used to fertilize oocytes *in vitro* and is the method that was used to conceive Louise Brown in the late 1970's. Our physician often recommended patients with only female-factor infertility or mild male-factor infertility to use INSEM. On the day of the patient's retrieval, the lab asked that a fresh semen sample be collected by the male partner in-office unless the patient is using donor sperm or a frozen vial of sperm, TESE or PESA sample. We always encouraged a fresh sample for IVF: however, if frozen sperm is being used for any reason, the vial is thawed the morning of the retrieval and can be processed using the same protocols as a fresh sample. Depending on the quality of the sample, modifications could be made to sperm washing/gradient protocols might be adapted on a patient-by-patient basis.

Typically, sperm used for INSEM were processed using either a swim-up or density gradient protocol at the clinic where I worked. The WHO also suggests using a density gradient rather than the swim-up method because gradients tend to yield better quality samples with higher concentrations—ideal for INSEM (WHO, 2010). Per our laboratory Standard Operating Procedures, after making initial assessments for a standard

semen analysis, we would load the sample onto a 45/90 gradient, centrifuged and washed twice using sperm wash media to remove all white blood cells, debris, and nonmotile sperm. The post-processing parameters we required in order to use INSEM for a patient were: 14M/mL concentration or greater, >40% motility, forward progression of 3+ or higher, and a minimum morphology score of a 4. If the sample decreases in quality after processing (rare, but does occur), the lab informs the physician that the sample is not good enough for INSEM and a more aggressive protocol may be necessary for fertilization—intracytoplasmic sperm injection [ICSI]. Consent forms for IVF at our facility had several options for fertilization that the patient could choose: INSEM only, INSEM unless the sample is not good enough, then use ICSI, 50/50 INSEM and ICSI, or ICSI only. If the patient has not signed off for ICSI under any circumstances but the semen sample does not meet the requirements for INSEM, our physician would contact the patient and discusses options. The patient could sign new consents permitting the use of ICSI and agree to pay the additional charges for that service, attempt INSEM with the current sample and expect few successes or a "no-fert," which would render their entire cycle a failure, or choose not to fertilize the oocytes and freeze them to be fertilized later. Most patients choose to allow ICSI under these circumstances or will freeze the oocytes for future use rather than risking no fertilization occurring with the current sample.

After the oocytes have spent 3-6 hours in the humidified chamber to continue maturation and the retrievals for the day were complete, the INSEM process could begin. Standard INSEM protocol requires that oocytes were left in their cumulus complex for fertilization—this more closely mimics how fertilization would occur *in vivo*. Our clinic also followed this method of leaving the cumulus complex attached for INSEM cases.

We would move the oocytes from their post-retrieval holding dish into a new dish that was labeled with the patient's name and date of birth and filled with warmed fertilization media. If the patient chose INSEM and the processed sperm met all of the qualifications for INSEM, all of the patient's oocytes would be exposed to sperm, regardless of their observed maturity. Depending on the concentration of the patient's sperm sample, we would add a volume of 1-15μL of processed sperm to the fertilization media to fertilize the oocytes. After adding the sperm, the dish is closed and returned to the incubator until the following day (16-20 hours later) to check whether fertilization has occurred. The following morning, we would perform a "fert check" and document how many oocytes fertilized overnight, and how many did not. Some unfertilized oocytes can be exposed to sperm the following day to attempt fertilization again, but most often the unfertilized oocytes ended up being discarded.

A fertilized oocyte is then referred to as an embryo, which continues to grow and divide over the course of several days. Embryos are not checked or moved into new dishes on a daily basis. It is standard to check embryos on day 1 for successful fertilization, then to leave them in the humidified chamber to divide until day 3 before being checked again. Our lab checked and graded embryos only on days 3 and 5, then every day until all the patient's embryos had been transferred to the mother, frozen, or discarded. Checking the embryos as little as possible avoids removing them from the humidified chamber very often. Having a set schedule of when to observe embryos also allows better scheduling in the embryology lab when there are many patients with embryos being cultured on any given day. Given the clinic where I worked saw such a

high-volume of patients each month, there were often over 100 embryos in the lab being cultured or prepared for transfer during our busy weeks.

By day 3 post-fertilization, the embryo should have reached the cleavage stage and have between 6-10 cells inside. Embryologists observe the embryos under a microscope, count the embryo's cells, and observe the overall quality of the embryo. We used an embryo grading chart to provide grades for the embryos and would document the number of oocytes with each grade. At this stage, an embryo should ideally have 8 uniform cells inside with little to no fragmentation or vacuoles. Depending on the number of embryos in the dish, sometimes day 3 embryos were moved into a new, clean dish to replenish nutrients as the embryos continue to grow. On day 5, we would give the embryos a second grade based on how much they have expanded, the quality of the inner cellular mass, and quality of the trophectoderm. Day 5 embryos should have two distinct layers: the outer trophectoderm that will form the placenta and inner cellular mass that will become the fetus.

Ideally on day 5 the embryo qualifies as a blastocyst, with many uniform, tightly packed cells and is beginning to hatch out of the zona. Starting on day 5, we would begin to freeze embryos for the patient to use in the future (see Figure 11). If a patient was having a fresh transfer, this is the point at which those embryos could begin to be transferred. However, as mentioned previously, the clinic where I worked did not perform fresh transfers often, so most embryos were frozen starting on day 5. Trophectoderm biopsy for genetic screening of embryos was also performed on day 5/6, a technique discussed in more detail in Chapter 5. By day 7, all the patient's embryos should be frozen or have been transferred—our clinic did not freeze embryos beyond day

7. After day 7, blastocysts require more nutrients to survive each day and cannot be maintained on culture media alone.

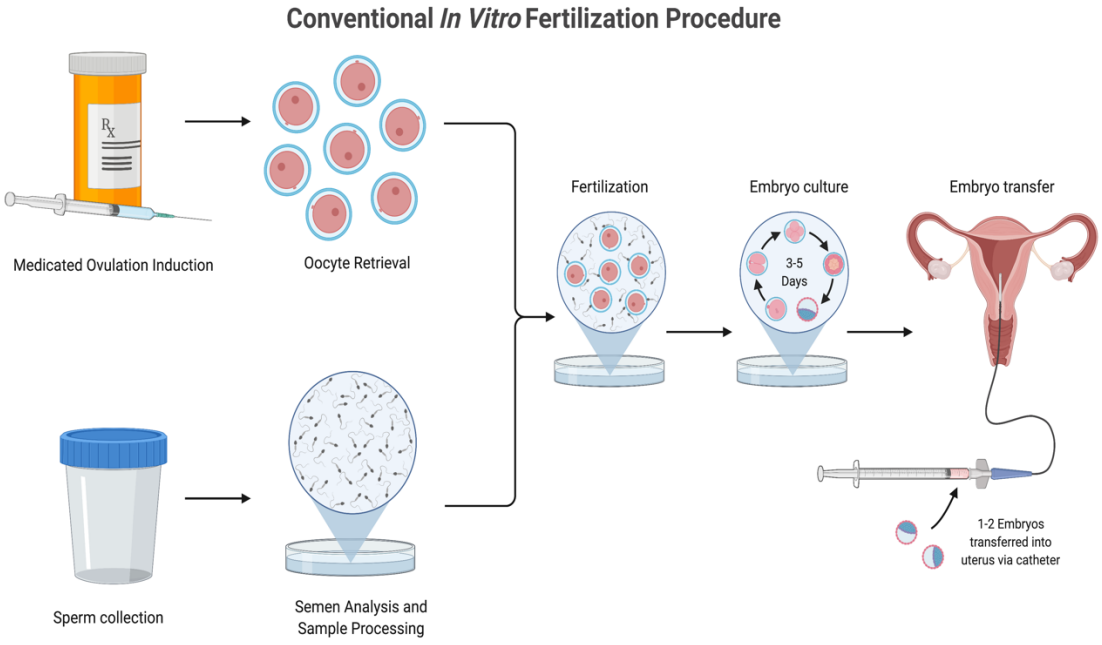

Figure 11. Conventional *In Vitro* Fertilization Procedure. Major steps of the IVF process are covered in this diagram, from ovulation induction to embryo transfer. Created with BioRender.com.

Intracytoplasmic Sperm Injection

In cases of lower semen quality, ICSI might be needed to manually inject a single sperm into the oocyte to increase the likelihood of successful fertilization. ICSI is used most often with patients who have few sperm in their ejaculate, sperm with low forward progression scores or very abnormal morphology. At our clinic, patients with known low sperm quality would be encouraged to sign consent forms to use ICSI only. Patients with mediocre-quality sperm who have a chance of qualifying to use INSEM may be told to

sign to use INSEM, with consent to use ICSI if the sample is not good enough. However, there were occasions where patients with no male-factor infertility at all choose to use ICSI over INSEM out of a matter of preference or under the assumption that a more expensive technique like ICSI will yield better results. The primary benefit of ICSI is that the highest-quality sperm are hand-selected by trained embryologists and are individually inserted into each oocyte—sperm that otherwise would not be able to successfully fertilize an oocyte in their own.

The first critical step of ICSI begins before sperm are even in the dish. Following the maturation period in the humidified chamber, oocytes being inseminated through ICSI must undergo a process called "stripping," which uses an enzyme called hyaluronidase to remove the sticky cumulus cells that surround each oocyte. At our clinic, stripping was performed one-hour post-retrieval. This allowed the oocytes to equilibrate in the media where they were being held before undergoing stripping, which is an abrasive process for the oocytes. When the oocytes were ready to be stripped in preparation for ICSI, we would remove them from their holding dish and place them in a separate dish containing several individual drops of warmed hyaluronidase diluted in culture media. The oocytes are mixed around in the hyaluronidase solution for a few seconds and the surrounding cumulus cells begin to slough off. The stripped oocytes are then removed from the hyaluronidase solution and rinsed in several drops of warm culture media to remove any trace of the enzyme before being returned to their holding dish. Stripping is a quick process that takes only a few moments and there is no set amount of time that the oocytes must spend in the solution—it is based entirely on how quickly the cumulus cells come off on their own. During the stripping process, the

oocytes are observed constantly under the microscope, because if the oocytes are left in the hyaluronidase solution for too long, the enzyme will begin to eat through the zona which can cause irreparable damage and potentially kill the oocyte.

After the cumulus cells have been removed from the exterior of the oocyte, we would return the oocytes to the incubator while the microscope is prepped for ICSI. Since oocytes are extremely sensitive to changes in temperature, osmolarity and pH, the less time they spend outside of the incubator and uncovered, the better off they are. There are two different needles used for ICSI—a blunt holding needle for the oocyte and tapered injection needle for the sperm. The side that each needle is on (left or right) is a matter of preference for the embryologist performing the ICSI procedure—however, the manufacturer specifies the angle for the needles' positioning. That must be done properly, or the needles will be more likely to break as they are moved vertically into and out of the dish. Angles for either needle can vary based on the manufacturer but typically range from 20º to 45º angles. The needles are connected by thin tubing to the microinjector which allows the embryologist to control the strength of suction or expulsion from each needle. Both needles are attached to the microinjector and brought into focus before moving on to the sperm preparation.

To prepare for ICSI, we would always add the sperm to the dish first—since oocytes are much more limited in quantity (compared to sperm) and are more sensitive to changes in temperature, we would only add the oocytes to the ICSI dish moments before we were ready to do ICSI. Using a micropipette, we loaded a small aliquot of sperm into an L-shaped droplet of media in the ICSI dish. Since sperm tend to swim along the outer edge of a droplet, the L-shape allows sperm to swim in different cardinal directions

which makes them easier to pick up with the needle. Under the microscope fitted with the microinjector needles, we look for the best quality sperm that are morphologically normal and are swimming rapidly in a relatively straight path. The best-looking sperm are picked up and deposited into a separate drop in the dish made of polyvinylpyrrolidone [PVP]. PVP is a viscous solution that decreases sperm motility, lubricates the injection needle, and prevents sperm from sticking to the inside of the needle and gives the embryologist greater control over movement of the sperm (Cooper Surgical, 2016). Once several sperm have been deposited into the PVP, they appear sluggish in comparison to their once-rapid movement in the L-bend of media. The sperm can then be immobilized by using the injection needle to quickly strike their tail (Patrizio et al, 2003). Immobilization is important for the ICSI procedure because sperm must be injected into the oocyte head-first. When loading, sperm are picked up by their tails, so they are expelled into the oocyte head-first. Immobile sperm are not dead, just stunned, and unable to turn around in the needle before injection.

Unlike with INSEM, only mature oocytes can be used for ICSI, so mature oocytes are loaded into a drop of media on the ICSI dish, leaving the immature and germinal vesicle-stage oocytes in the holding dish. Under the microscope, one oocyte is picked up at a time using the holding needle—the suction of the microinjector holding onto the oocyte is firm enough that the oocyte will not slip during injection, but light enough to not rupture the zona. I was trained to position the oocyte so that its polar body was positioned near 6 or 12 o'clock—as far away from the injection site as possible without being directly under the suction of the holding needle, which is demonstrated in Figure 12 below. With a single sperm loaded tail-first into the tip of the injection needle, the

needle is inserted through the zona, and the cytoplasm of the oocyte called the "oolemma" is aspirated just until it ruptures so the sperm cell can be injected inside. Most embryologists aspirate and expel a small portion of the oolemma once the sperm has been deposited to mix the sperm with the oocyte's cytoplasm—this ensures that the sperm is not stuck to the tip of the needle and will not be removed when the needle is withdrawn.

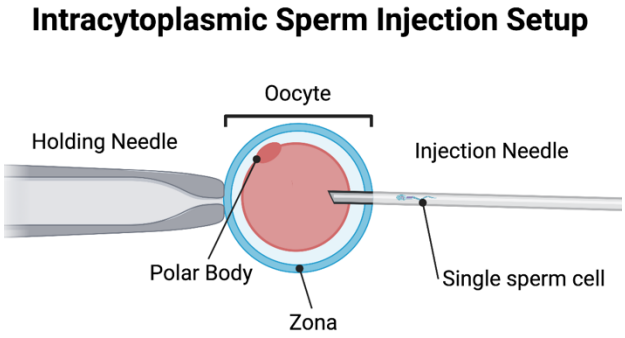

Figure 12. Intracytoplasmic Sperm Injection Setup. Created using BioRender.com

Once the injection is complete, the embryologist rolls the oocyte out of the way so ICSI can be performed on the remaining oocytes. As an embryology trainee, I would only work with a couple of oocytes at a time to prevent the oocytes from being out of the incubator for too long, while the more senior embryologists could perform ICSI much faster. Oocytes that have been fertilized via ICSI are then moved to a new labeled dish of media to culture for another 5-7 days until transfer or vitrification. The culturing process of ICSI and INSEM embryos is identical, with embryo/blastocyst grading is performed on days 3 and 5, possible trophectoderm biopsy on day 5/6, and potential vitrification of the blastocysts between days 5-7 until all blasts have been transferred, frozen or discarded.

Oocyte/Embryo Vitrification and Thawing

Vitrification is a process of rapid cooling used to quickly cryopreserve embryos and oocytes. This is not considered an ART or even a fertility treatment, but is a frequent protocol used in fertility clinics that merits discussion. Since only one or two embryos are transferred back into the uterus at a given time, any additional embryos must be cryopreserved and stored in liquid nitrogen until they can be transferred. The process of freezing embryos at the end of an IVF cycle has been performed for many years—with some patients returning 5-10 years after their cycle to transfer a previously-frozen embryo. Autologous oocyte banking is a much newer service initially used to help patients undergoing cancer treatment to preserve their fertility. Oocyte cryopreservation has recently gained popularity for patients who would like to preserve oocytes during their younger years so age-related decline in oocyte quality does not impede conception (Mertes & Pennings, 2011). Oocyte cryopreservation has also become a method for transgender patients to preserve their ability to have biological children even after hormone-replacement therapy and transitioning (Mitu, 2016).

The overall process of vitrification is the same for both—the oocyte/embryo is slowly moved out of the media into increasing concentrations of equilibration solution, then out of equilibration solution into increasing concentrations of vitrification solution until the oocyte/embryo is in 100% vitrification solution—a process that takes roughly 15 minutes from start to finish per our protocol at the clinic. After reaching 100% vitrification solution, the oocyte/embryo is loaded onto a labeled cryo-straw and dipped into liquid nitrogen and flash-frozen for long-term storage. If frozen and stored properly, these embryos and oocytes can be stored indefinitely using the vitrification technique.

The only differences between vitrification for oocytes versus embryos is that oocytes must be stripped one hour after the retrieval if they are being vitrified and not fertilized.

Our protocols for thawing oocytes and embryos utilized different solutions than vitrification, but still followed a similar process. Thawing begins in 100% thawing solution before introducing increasing concentrations of diluent solution. Once the oocyte/embryo is in 100% diluent solution, washing solution is gradually introduced until the embryo can be moved into culture media. The process of thawing embryos and oocytes also took roughly 15 minutes from start to finish, at which point an embryo would be used for transfer, or we would begin the INSEM or ICSI procedure to fertilize the thawed oocyte.

Conclusion

Many different protocols and techniques are utilized in fertility clinics today to assist patients with starting or expanding their families. The multitude of available methods for both stimulation and conception gives patients more autonomy in how invasive and involved their care will be. Regardless of which method or treatment a patient undergoes, reproduction at the fertility clinic is a collaborative effort between numerous participants or "key players." Each of these participants plays a unique and essential role in the reproductive process. In this chapter, I described the roles, responsibilities, and contributions of the key players at a fertility clinic: physicians, nurses, medical assistants, patients, and laboratory staff.

I have emphasized the role of laboratory staff in particular, as patients typically have fewer interactions with laboratory staff compared to other staff at the clinic, and my expertise primarily lies in my laboratory training at the clinic. Despite having fewer

interactions with patients as a member of the laboratory staff, I noticed that some patients visiting the clinic expressed genuine interest in understanding the work of the andrologists and embryologists in the lab. Most laboratories housed within fertility clinics are out of view from patients and visitors at the clinic, so patients wonder how we were able to create embryos for patients who had tried for years to conceive naturally and were not successful. Through these patient interactions, I realized that the inner workings of the lab are not communicated well to patients. Patients wanted to know what was going to take place in the lab either before or during their treatment—how these technologies could do something that was otherwise impossible.

Disclosing these laboratory processes can help patients feel better informed about what is happening to their sperm, oocytes, and embryos during the fertility treatment process. Patients understanding workflow in the lab can also benefit the clinic, as patients become more knowledgeable about the techniques, skills and supplies that are involved in the preparation of samples, insemination of oocytes, embryo culture/biopsy/cryopreservation, and all other tasks that are carried out in the lab during a patient's cycle. If patients are better informed about what the laboratory is responsible for, I believe clinics can justify some of the costs associated with receiving treatment more easily. Few clinics itemize laboratory costs when advertising a fertility treatment package, so the cost of maintaining a clinical laboratory and the volume of supplies utilized goes largely unnoticed.

Cost aside, ARTs have undoubtedly changed the way reduced fertility is viewed from a clinical standpoint, as well as in the eyes of individuals struggling to conceive. The implementation of ARTs in reproductive medicine as a means to circumvent reduced

fertility has also paved the way for ethical dilemmas involving misuse of these technologies, and the use of ARTs for unintended purposes. But how far is too far? Have we reached the limits of what the technology *should* be used for, even if we *could* use it for more? What ethical challenges have arisen in the fertility field to date, and have they been addressed or are these issues still prevalent in the field? These questions will be addressed in Chapter 5, Ethical Dilemmas in Fertility.

CHAPTER 5

ETHICAL DILEMMAS IN FERTILITY

Synopsis

How far is too far? Just because we can, should we? Where do we draw the line? These types of questions routinely arise in the field of reproductive medicine, especially with the implementation of novel treatments and therapies. Since its inception, the use of Assisted Reproductive Technologies [ARTs] in fertility medicine has been met with challenges. Initially, terms such as "playing God" and "test tube baby" were used to describe the use of ARTs in reproductive medicine and the children who are born as a result. While the societal acceptance of ARTs has improved over the years, the field still faces many unresolved ethical issues. Although there are many ethical controversies in assisted reproduction that have been highlighted more recently in the news such as using CRISPR gene editing to "fix" or "enhance" an individual's genetic makeup, sex selection, savior siblings, or embryo ownership/disposal, this chapter will focus on a set of ethical dilemmas encountered within the clinic that I worked. In this chapter, I discuss three of the prominent ethical quandaries that I observed while working in the field of reproduction. These issues concern profiling and anonymity of sperm donors, oocyte donor compensation, and the ramifications of specialized forms of pre-implantation genetic testing, incorporating including personal anecdotes and observations. I describe the historical context of each issue, discuss what classifies it as an "ethical dilemma," and relate these situations back to my time work at the clinic.

The Fertility Business

Significant growth of the fertility sector has led to rapid development and implementation of new assisted reproductive technologies [ART]. The net worth of the global fertility market in 2020 is estimated to be around $20 billion but is expected to double by 2026 to roughly $41.4 billion (Tomkins, 2020). To date, market growth of the fertility sector has largely been attributed to declining fertility rate, improved access to local fertility specialists, and increased success of IVF cycles due to the implementation of ICSI (Tomkins, 2020). Going forward, the use of genetic testing of embryos, more widely available at-home fertility testing, and use of artificial intelligence in fertility medicine are projected to have a positive impact on the fertility industry as well (Nayot, 2020).

Fertility clinics function as a business—providing consultation, diagnosis, and conception service packages for patients. In the U.S., businesses are typically regulated through various state and government-made laws that outline expectations with which the business must comply. These can include laws such as how businesses tax their services, how they must pay and provide benefits to their employees, ways to protect workers from discrimination, workplace safety laws, and anti-trust laws that prevent businesses from reducing competition through forming monopolies or fixing prices (USAGov, 2021).

Business laws have been implemented for decades to protect customers, employees, and the business itself. However, fertility clinics (as a service-providing business) in the U.S. have been described as underregulated, and many of the existing guidelines put in place by federal and industry regulatory agencies are not strictly followed, even though they should be (Ollove, 2015). There is a significant lack of

regulation in fertility medicine in the United States across individual states, counties, and clinics regarding the way that data is reported from clinics to reporting agencies, how specimens are stored both short and long-term, and how laboratory errors and inaccuracies are handled for re-accreditation purposes (Fox, 2018). In an interview conducted in 2015, executive director of the Center for Genetics and Society Marcy Darnovsky described the regulatory environment of the United States fertility industry as "the Wild West" (Ollove, 2015). This idiom refers to the rough, lawless nature of early pioneering times in the United States—a rather uncomplimentary description of how fertility medicine is monitored. Dr. Dov Fox of the University of San Diego's School of Law remarked that:

> Few… specialties in the United States are as opaque as assisted reproductive technology. ART operates free of regulation about serious and preventable kinds of errors that might be called ART "never events": the destruction, contamination, misdiagnosis, and switching of materials that cannot be chalked up to inevitable slips of hand or reasonable lapses in judgment. Elsewhere in health care delivery, these kinds of mistakes…are publicly reported by mandate…But no system exists to track similar such transgressions when they take place at fertility clinics, sperm banks, egg vendors, or surrogacy agencies (Fox, 2018).

Labs located inside fertility centers follow a few common procedures of operation. At minimum, andrology and embryology laboratories are required by the U.S. federal government to be certified by the American College of Pathologists, called "CAP certification." Additional certification through the Clinical Laboratory Improvement Amendments [CLIA] is recommended but not required. These laboratory-specific

accreditation programs are designed to promote better practices for specimen handling and laboratory techniques but provide no protection for patients (customers) from accidents or negligence involving the handling of their specimens in the lab (CDC, 2021a; College of American Pathologists, 2021). Laboratories are also required to report general cycle data so annual reports can be made to calculate the number and type of cycles that were conducted, and report live births from those cycles (CDC, 2021a).

The fertility clinic where I worked was both CAP and CLIA certified. Members of the andrology and embryology laboratories were responsible for ensuring that we maintained compliance with the regulations put in place by both accreditation programs. Our laboratory director at the clinic was off-site but made trips to our clinic every few months to ensure that we were keeping up with records, saving and storing patient information in compliance with the Health Insurance Portability and Accountability Act, properly conducting daily quality assurance checks, and numerous other requirements held by the CAP and CLIA programs. Since our laboratory conducted blood serum immunoassays on-site using an immunoassay analyzer (the TOSOH AIA900), we had additional biochemical proficiency testing from CAP. This was to ensure that our analyzer was calibrated properly, and that we were keeping up with the required annual inspections and preventative maintenance.

Upkeep and accreditation of the laboratory is one aspect of the work carried out by the clinic. The interactions between physicians and patients and the overall ART process is “self-regulated” by The American Society for Reproductive Medicine [ASRM]. The ASRM does provide guidelines for health and laboratory professionals working in reproductive medicine but does not reprimand or in any way sanction those

who do not adhere to those guidelines (Ollove, 2015). Rather, ASRM markets its membership as a tool for continuing education, networking, and receiving discounted rates on annual conferences and journal subscriptions. General lack of accountability within U.S. fertility clinics has created discrepancies in diagnosis, treatment plans, costs, data reporting, and quality of care across clinics (IVF Authority, 2020).

Absence of regulatory oversight in fertility medicine in the U.S. has allowed for rapid growth. However, there are far more disadvantages to this "fertility free-for-all" than there are advantages. The lack of consistent and firm regulation of the industry has allowed for mishaps in the clinic and during annual reporting that go unnoticed more easily and has allowed numerous ethical dilemmas to arise regarding current use and future implications of ART. For the three prominent ethical issues I explore, namely profiling and anonymity of sperm donors, compensation of oocyte donors, and use and implications of genetic testing performed on embryos, I begin with the history of how the practice or technique was developed and how it became a common occurrence in the clinic today. After the historical context, I discuss why each of these three examples constitutes an ethical dilemma. I accomplish this through the description of scenarios that illustrate why lack of regulation has led to these ethical issues. The scenarios that I analyze in this chapter come from both literature review, news in reproductive medicine, and my own experience in the clinic—demonstrating that the ethical implications of lack of regulatory oversight in fertility medicine has been and continues to be problematic for clinics and patients alike.

Sperm Donation in the United States

The development and implementation of sperm donation practices in the U.S. has a rather problematic history. The first reported case of sperm donation dates back to the 19th century—long before the convenience of online sperm banks, donor selection guidelines and semen quality standards were even imagined. In this section, I begin with the development of sperm donation as an element of fertility medicine. This includes documenting the history and initial ethical issues in sperm donation practices in the U.S., using notable cases that have been publicized over time. After describing the complicated historical framework that surrounds sperm donation practices today, I describe how legislation (or lack thereof) has led to the development of additional ethical dilemmas in the practice. Then, I address the more modern ethical dilemmas of sperm donation in the U.S. by assessing current donor profiling practices utilized by clinics and sperm banks. I analyze the standards these facilities set for their donors and compare how banks describe what makes an "ideal" sperm donor. After establishing "ideal" standards for donors, I describe the ethical implications of profiling sperm donors and differentiate between standards for donors versus standards for sperm, which are two very different measures of donor quality.

This section also includes a personal account from my clinical training of how donor sperm is handled and processed in an IVF laboratory, highlighting the importance of anonymity and how clinics protect sperm donor anonymity in the clinic. The final ethical issue I cover in this section is a more current issue promoting sperm donation on the grounds of remaining anonymous. I describe the meaning of anonymity and

disclosure of donor information in the clinic and discuss how popularization of consumer genetic testing can easily compromise sperm donor anonymity.

In 1884 at Jefferson Medical College in Pennsylvania, a husband and wife sought advice from Dr. William Pancoast on how to conceive after months without success. The wife underwent an initial physical exam from Dr. Pancoast, who found no reason for her infertility. Dr. Pancoast then conducted a medical examination on the husband and determined that there was no sperm in his ejaculate, and diagnosed him as azoospermatic (Hard, 1909). Rather than disclosing the husband's unfortunate results, Dr. Pancoast asked that the wife return for a more intensive examination. The female patient was anesthetized using chloroform, and unbeknownst to the couple, the wife was inseminated using semen from the "best looking member of the class"—a medical student (Gregoire & Mayer, 1965). When the wife became pregnant, the husband questioned how this was possible, and Dr. Pancoast divulged the true father of the child. The husband asked that his wife never find out that the child was not related to him (Gregoire & Mayer, 1965). This incident was kept a secret for over two decades until Addison Hard, one of the medical students present for the procedure published a letter in *The Medical World* in 1909 following the death of Dr. Pancoast. Hard's letter revealed all the details of the first reported insemination using donor sperm (Hard, 1909; Gregoire & Mayer, 1965).

Despite success of the procedure, there was much controversy in the medical community over Dr. Pancoast's inadequate medical disclosure to the patients, and the extreme lapse in judgment regarding his encouragement of one of his own students to serve as a sperm donor. Decades following the publication of Hard's letter in 1901, few instances of sperm donation were reported. It was not until 1945 that London obstetrician

Dr. Mary Barton and her husband Dr. Bertold Wiesner revisited the concept of sperm donation for artificial insemination—specifically for women whose husbands had an incurable form of infertility (Barton et al., 1945).

In Barton et al.'s study, donors were kept completely anonymous—the donor was never described to the couple receiving the sperm to prevent future identification of the donor, and the same level of secrecy was promised for the receiving couple (Barton et al., 1945). The intended parents were only guaranteed that donors had been meticulously selected by Drs. Barton and Wiesner, and that the donors were of good health. Additional selection criteria for donors remained undisclosed to patients. In order to donate sperm, men were required to have reasonable sperm count/quality, two legitimate healthy children prior to donation, and aged 30 to 45 (Barton et al., 1945). Barton and Wiesner then chose a donor from their small pool of hand-selected donors that they believed best matched the intending parent's race, blood type, intellectual achievement, and social competence, which led to over 1,500 babies being born as a result of the program (Fricker, 2012; Smith, 2012).

Decades later, the study conducted by Barton and Wiesner began to unravel. Two men who were conceived through the sperm donation program at the clinic discovered through DNA testing that they had the same father—Dr. Wiesner (Fricker, 2012). Wiesner was making sperm donations to his own program, and there is speculation that he fathered around 600 children out of the 1,500 babies born through his clinic (Smith, 2012). Consumer genetic testing has also connected over 100 children to a British neuroscientist that participated in the program and 150 to an American donor from the clinic as well (Fricker, 2012).

Similar stories have surfaced over time regarding other doctors using their sperm to inseminate infertile patients. Dr. Peven, a physician who practiced in Detroit in the 1940's fathered hundreds of children during his time as a fertility specialist (Parry & Harvey, 2020). A second noteworthy case concerns Virginia physician Dr. Cecil Jacobson, who reportedly helped to conceive between 15-75 children during his time as a physician in the 1980's (Parry & Harvey, 2020). In 2014, a third case was discovered using DNA testing, which revealed that Indiana fertility clinician Dr. Donald Cline used his sperm to impregnate patients at his clinic, resulting in the birth of roughly 50 babies between 1970-1980 (Zhang, 2019). Finally, in an interview published by USA Today in 2021, consumer DNA testing showed Dr. Martin Greenburg, a former fertility specialist in New York City had used his own sperm to inseminate one of his patients in 1983 (McCoy, 2021).

These disturbing and unethical cases of "fertility fraud" have come to light because of advancements made in DNA testing for the public. The ethical issue in this case is failure to disclose the true source of sperm, lying about using sperm the patient purchased and requested to be used, and the physician committing what could be considered medical rape on the patient by using his own sperm without receiving consent from the patient to do so. While DNA testing has helped to curb this kind of medical malpractice going forward, DNA testing has resolved some ethical issues, but created others at the same time, particularly for sperm donors that donate through licensed sperm banks that wish to remain anonymous—a topic that I will discuss in greater detail in section 5.3.4.

Legislation of Sperm Donation in the United States

Sperm donation in the U.S. also has a complicated legislative history that has shaped the way sperm donors are viewed, treated, and protected. Understanding the way that sperm donor legislation has changed over time is important for setting the stage for discussing how sperm donors are profiled and protected in the U.S. The legal framework of sperm donation in the U.S. was nearly nonexistent until 1954 (California Cryobank, 2021a). In most states, children born through donor insemination were declared illegitimate, regardless of whether the husband consented to the procedure (California Cryobank, 2021a). The claim that donor children were illegitimate regardless of the husband's consent was dismissed first by the state of Georgia in 1964, legitimizing children conceived through donor insemination permitting both the husband and wife gave consent (California Cryobank, 2021a). Several other states began to follow suit, and in 1973 the Uniform Parentage Act was drafted by the National Conference of Commissioners on Uniform State Laws and was approved and recommended for all states (Krause, 1974). The Uniform Parentage Act of 1973 established the early framework of sperm donation, stating that if a couple equally consented to use donor sperm to conceive a child, the law will treat the husband as the natural father of the child (California Cryobank, 2021a). The act also protected donors from being financially or legally obligated to provide for children conceived using donated sperm (Krause, 1974).

Outlining the rights, roles and responsibilities for each party involved in conceiving a child using donor sperm was a monumental step for regulation of fertility medicine—until that point, there was little provision for children conceived using donor sperm. Still, the Uniform Parentage Act began with vague guidelines for "who is the

father" that excluded situation of divorce, parental rights of non-marital fathers, unmarried women, or women using donor sperm not under the supervision of a licensed physician (Luetkemeyer & West, 2015). The act only applied to a single scenario: married women using donor sperm inseminated by a licensed physician. In any other case, the paternity of a child conceived using donor sperm could be drawn into question. The first draft of the act adopted by some states was not comprehensive nor inclusive enough given the diverse family dynamics present across the country and required revision.

The first revision of the Uniform Parentage Act attempted to provide clearer descriptions of child paternity to delineate legal responsibilities for children conceived using donor sperm. The 2002 revision of the Uniform Parentage Act removed the requirement of insemination by a licensed physician and prevented the donor from suing for parental rights later on, but did allow sperm donors to fight for custody if they lived with the child they helped to conceive within the child's first two years of life (Luetkemeyer & West, 2015). The Uniform Parentage Act was amended again in 2017 to add additional legislation to protect children conceived through ART, surrogacy agreements, unmarried couples, same-sex couples, and to give legal rights to parents not biologically related to the child (Parness, 2018). However, since adoption of the Uniform Parentage Act was a *recommendation* for states, not a requirement, this led to a lack of congruence in sperm donor rights and parental rights between states, which was further complicated by cases where donors and intended parents lived in different states.

With discrepancies between states on determining child paternity, as well as who holds legal guardianship over a donor-conceived child, ill-defined legislation still left

loopholes for sperm donors to be pursued by the intended parents for child support in certain states, and under certain circumstances. In 2009, a man in Kansas, William Marotta, donated sperm to a lesbian couple he found through an online advertisement. The couple performed the insemination procedure at home by themselves and a healthy baby girl was born to the couple (Pekarsky, 2016). The couple separated the following year and the birth mother had primary custody of the daughter. While seeking financial assistance for herself and her daughter after becoming ill, the birth mother disclosed the name of the sperm donor as the biological father of the child on her Medicaid application. The state of Kansas pursued Marotta for child support because the sperm was used at home and not at a licensed fertility clinic, which in the state of Kansas made Marotta the legal father of the child (Pekarsky, 2016). Despite Marotta having no contact with the couple or child since his donation, the judge presiding over his case ruled in 2014 that Marotta was financially responsible for the child since the sperm was used at home and not at a clinic (Pekarsky, 2016). The state of Kansas had not adopted the Uniform Parentage Act that would have prevented Mr. Marotta from being considered the child's legal or financial responsibility in any way. In 2016, however, Marotta's case was reopened, and the same judge who ruled against him in 2014 changed the case ruling and released Marotta from his obligation to support the daughter he helped to conceive (Pekarsky, 2016).

Protection of Donors Through Sperm Banking

To circumvent the various risks and complications associated with donating sperm, sperm banking facilities began appearing in many states. Sperm banks serve as intermediary enterprises that help both donors and recipients. Donors are often financially

compensated for each donation they make to a sperm bank. Depending on the sperm bank, donors can be compensated anywhere between $20-$125 per sample provided (Cryos International, 2021; The Sperm Bank of California, n.d.). The identity of sperm donors is protected by these banks through "anonymous" donation—the clinic associates vials of sperm with a serial number or fake name, but any personally identifying information is kept confidential.

Donors can choose to have their identity released to children conceived using their sperm once the child becomes a legal adult at the age of 18. If donors choose the "identity release" option, the sperm bank will reach out to the donor child at the appropriate time and provide them information about their donor (Cryos International, n.d.). Regardless of whether a donor chooses to donate anonymously or have their identification released to donor children later in life, donors legally waive their parental rights to any children conceived using their sperm (Cryos International, n.d.). By doing this, intending parents are prohibited from seeking child support from the donor, thus preventing cases such as Mr. Marotta's legal battle over donor child support in 2009 (Cryos International, n.d.).

Intended parents are also protected by sperm banks. The signed legal document required prior to donation of sperm prevents donors from seeking custody of any children conceived using their sperm. Purchases made by patients through a sperm bank are also backed by a quality guarantee. When thawed, the sperm bank guarantees that the sperm frozen in the vial will meet a set of parameters (that they establish), regarding sperm motility, total motile sperm count, and volume. These parameters vary depending on the type of vial the patient ordered, but nevertheless, the bank has written quality standards to

ensure that if a vial thaws poorly, the patient can get their money back or receive credit to purchase a new vial from the same donor, or a different donor if the patient prefers. Sperm banks also establish their own standards for the "quality" of donors they will allow to donate to their bank. Banks provide patients with reports on the donor's medical and sexual history, age, interests, occupation, education, and many other qualities so patients can make informed decisions on who the sperm donor will be for their child (Xytex, 2021). However, while these criteria are beneficial for the patient to know, they can also exclude certain men from donating sperm, which will be discussed in section 5.3.3.

My experience in recordkeeping and the process of documenting sperm storage is limited to my experience at a single fertility clinic. However, many clinics use cryotanks and cryochambers to store samples, so the method should be the same. At the clinic where I worked, embryos and sperm were kept in separate tanks—there were no tanks that contained a mixture of vials of frozen sperm and vials of frozen oocytes or embryos. When our clinic received a tank of donor sperm for a patient from an outside lab, we would retrieve the packing slip from the donor sperm package and verify that the donor sperm ID matched the receipt that the patient uploaded to our Electronic Health Record System. We also checked that the vial type matched what the patient ordered. For example, if the patient ordered a vial of sperm processed for an IVF, but received a raw IUI vial instead, we would contact the patient and inform them that the wrong vial was sent and would either refuse the package and send the vial back or contact the bank and receive a price adjustment for the type of vial received. From my experience, we never encountered a vial that did not match the patient's chosen donor ID, nor did we receive a vial that was processed in a way the patient did not specify.

Figure 13. Long-Term Cryostorage of Sperm and Oocytes at the Fertility Clinic where I worked. Photo Credit: Shelbi Peck, 2019.

After confirming the vial ID, patient ID and vial type, I would prepare to store the sperm in our facility (pictured in Figure 13 above). We stored sperm on a labeled metal cane (tube holder) that had space to hold up to six vials of sperm. Each cane was labeled with a colored metal tab at the top that would stick out of the liquid nitrogen. When we received a vial of sperm, we would write the donor ID and the intended parent on the tab, so we did not have to remove vials of sperm from the liquid nitrogen just to check the ID. Doing this helped to prevent premature thawing of vials of sperm when we opened the cryo tank. When storing sperm, I would find an open space in a cryo tank for donor sperm and record the tank and canister number that the cane would be placed in, as well as the color of metal tab we used to label the cane, and what was written as the identifying information on the tab, which was typically the patient's first initial, last name, date of birth, and donor ID. Once the sperm was safely stored in the tank, I would scan the packing slip with the vial location written on it and upload it to the patient's

online health portal. I would reach out to the patient via our telehealth communication platform, Klara, to inform the patient that their sperm had arrived and that the packing slip for their order has been uploaded to their health portal. For the laboratory's records, I would also add the donor ID and number of vial(s) that we received in that shipment to our cryo inventory spreadsheet to keep track of who we have vials for, and how many remain.

Sperm Banks and the Ethics of Donor Profiling

Introduction of sperm banks in the U.S. seemed to provide much-needed continuity and provision for the process of sperm donation. However, there are still significant flaws in the procedures and ethics of how sperm banks are operated, starting with the way donors are screened prior to approval for donation. The screening process begins with basic age and health requirements. Across five major sperm banks in the U.S.; California Cryobank, Seattle Sperm Bank, Fairfax Cryobank, Phoenix Sperm Bank and Cryos International, accepted donor age ranges from 18 to 44. All banks require that donors have no personal or family history of infectious disease or genetic disorders, and the donor must consent to more thorough genetic screening if they pass the initial screening. California Cryobank, Seattle Sperm Bank and Fairfax Cryobank explicitly require that applicants have graduated from (or be currently enrolled in) college to be considered for donation and must disclose their field of study (Seattle Sperm Bank, 2021). This information is provided to patients who are looking for a sperm donor, so they can choose a donor with a similar education level and field of study to their partner. Two clinics also have minimum height requirements for their donors: 5'7" for Cryos International donors, and 5'8" for California Cryobank donors (Cryos International, n.d.;

California Cryobank, 2021b). Some of the standards that sperm banks use to initially filter out potential donors are discriminatory—specifically height and education requirements which may prevent perfectly healthy men from donating sperm. By restricting donors based on these parameters, banks have essentially labeled certain men undesirable based upon presence of physical traits they cannot control and by labeling non-college graduates inadequate for reproduction.

If the potential donor meets all the preliminary requirements, donors are then asked to fill out comprehensive questionnaires about themselves which are used as a second round of screening and to build up their "donor profile." Applicants are asked questions that range from hobbies, interests and aspirations to the donor's religion, astrological sign, alcohol use, and whether they have tattoos or piercings (Fairfax Cryobank, 2020). Patients use basic profiles to find a donor that most closely aligns with their beliefs or desired traits/characteristics. Sperm banks list prices for donor sperm on their profile, often with increasing preparation and price such as: At-Home Intracervical preparations, IUI-specific vials, or IVF vials. One vial of sperm can range from $400 to over $1,000 depending on the bank and preparation of the vial. Extended information packages are also offered to patients for an additional cost which can include supplemental information about the donor such as audio recordings of the donor, childhood and adult photos, creative essays/drawings/recipes/songs produced by the donor, or a completed personality test. Fairfax CryoBank even offers a face-matching service that uses artificial intelligence to find donors that resemble their partner, themselves, or relatives. While these supplementary pieces of information may be ideal for couples trying to be incredibly thorough in their selection of a sperm donor, this can

lead to identification of a donor. The donor may not want to be identified until the child reaches adulthood or may not have wanted to be known at all. Providing prospective parents with information that may compromise donor anonymity can lead to a breach in the agreement made between the sperm bank and confidential donor. Clinics and sperm banking services should disclose that their practices and policies that benefit patients may compromise their anonymity and discuss implications of this with prospective donors. Omitting this information from patients should be considered wrong—especially for the sake of generating a small profit on top of what the clinic already receives as payment for a single vial of sperm.

Compromising Sperm Donor Anonymity

Many sperm donors only feel comfortable with their decision to help others conceive using their sperm because they can remain unidentified. The standards that sperm banks use to screen potential donors are already exclusionary and can discriminate against donors who possess traits that banks perceive as undesirable—even for characteristics that are not under the donor's control. Furthermore, banks that allow patients to pay for access to materials that may reveal the identity of the donor presents a serious threat to donor anonymity. While not all banks hide donor information behind a pay wall, placing a price on a donor's anonymity should not be permitted from any sperm banking facility. In conjunction with the questionable codes of conduct within sperm banks, loss of donor anonymity is becoming a more prevalent concern with the recent uptick in consumer genetic testing.

In a study conducted in 2019, it was estimated that over 29 million consumers had their DNA tested and their genetic information stored in databases through companies

such as AncestryDNA and 23andMe (Regalado, 2019). These companies use genetic information to build large family trees that show relatives across generations that may be living in different areas of the world. As more individuals have their DNA tested for various reasons, the database can connect individuals more easily. As the number of individuals missing from the database shrinks, so does the capacity to remain anonymous. For anonymous donors, this means that one of their close relatives who has their DNA stored in these databases can unknowingly expose the donor's identity in the process. Donors need to be informed that anonymity cannot be guaranteed anymore due to widely available consumer genetic testing. While donors are still protected from legal responsibility of any children conceived using their sperm, the lack of privacy may be off-putting for men considering donation in the future. With fewer donors willing to provide sperm samples to banks, this could lead to price-gouging of existing vials of sperm, or banks that offer men more money to donate repeatedly, which can become exploitative.

DNA testing has also revealed regulatory deficiencies within sperm banks regarding sperm use. Within the U.S. there is no legal limit for the number of children that can be conceived using a single donor. Practice Committees of the ASRM and SART jointly recommended a guideline for donor use in 2020 that suggests there should be no more than 25 children born from a single donor per 800,000 individuals in the population. Despite this guideline, sperm banks are not required to perform follow-ups with patients to record pregnancy outcomes, so this recommendation is largely disregarded in practice. As a result of insufficient reporting, there is no current statistic on exactly how many births using donor-sperm have occurred. Over the past several years, numerous cases

have been reported of donor children finding out they have dozens of half-siblings, thanks to consumer genetic testing (Cha, 2018).

Parents of donor children and donor children themselves are significantly impacted by loss of donor anonymity. This may allow parents of children conceived using donor sperm to identify their sperm donor more easily, but this has a much larger impact on the resulting children. A significant proportion of donor-conceived children are not aware that they were conceived using someone else's sperm. In a longitudinal study conducted by Golombok et al. in 2012, the psychological adjustment of children conceived through sperm donation was documented alongside the adjustment of their parents. Parents of donor children stated that they withheld information on their child's paternity out of fear that their children would resent them or their decision or would want the donor to be involved in their life, which might disrupt their current family structure (Golombok et al., 2012). As a result of parents withholding this information from their children, there is also concern that children may be traumatized when they learn about their donor-conceived status (Harper et al., 2016).

The overall definition of anonymity remains the same—it indicates a person's need for privacy (Pennings, 2019). In cases of sperm donation, this definition also encompasses the individual's desire to be omitted from the family dynamic and legal obligation to provide for a donor child. However, given the widespread interest in consumer genetic testing, there is a critical need for guidelines to be established in fertility clinics and sperm banks on how genetic information is shared with patients, as providing the option of donating sperm anonymously may not be realistic anymore.

Oocyte Donation in the United States

Addressing infertility in women is much more complex given the long-term role female reproductive organs play in the conception, gestation, and birth of a child. Compromised reproductive health in women can impact all stages of pregnancy and even cause complications during birth. For women with increased maternal age, diminished ovarian reserve, severe genetic disease, or fertility lost through chemotherapy and radiation, conception using their own oocytes may not be possible (Baetens et al., 2000). Until recently, women experiencing these severe forms of infertility were unlikely or unable to become pregnant and either remained childless or grew their families using adoption services.

Globally, the U.S. adopts the greatest number of children each year—out of 260,000 annual adoptions worldwide, over 127,000 were reported from the U.S. alone (United Nations, 2009). Adoption can be viewed as filling a need that already exists—there are children of all ages in foster care across the country who are awaiting a family to adopt them. For couples who would prefer to adopt very young children, there is also the option of infant adoption versus child adoption. Adoptive parents can choose the degree of communication they would like to have with the child's biological parents, if any (Acosta, 2013). Biological parents forfeit their legal guardianship over a child they give up for adoption, making the adoptive parents the child's new legal guardians (Acosta, 2013). Even with legal provisions that protect adoptive parents, adopting a child comes with unique circumstances that differ from "traditional" parenthood. The adoptive mother will not experience pregnancy, childbirth, or breastfeeding, and neither adoptive parent will be genetically related to the child. Social stigmas surrounding adoption

presented through popular culture and media have also negatively impacted the way society views the adoption process (Wegar, 2000). Families created through adoption can be viewed as not real, and adopted children can be portrayed as less desirable, problematic, and even maladjusted (Wegar, 2000). Both the biological realities of adoption and invented "downsides" can sometimes lead to couples refraining from adopting children, even if that is their only means for building their family.

For decades, adoption has been the only choice for women experiencing severe infertility due to low oocyte quantity or quality to build their families. However, increasing acceptance and effectiveness of IVF services following the birth of Louise Brown in 1978 encouraged scientists and clinicians to explore additional applications of IVF. By this time, donation of sperm was more widely accepted and practiced in the U.S., and several sperm banks were in operation across the country, providing semen samples to couples in need of a donor. But what about patients in need of oocytes? Collection of sperm for donation is undoubtedly an easier process than retrieving oocytes for donation, but the technique for retrieving oocytes was already in place for IVF, so why not use the same process to retrieve oocytes to donate to another patient?

In this section, I document the history of oocyte donation practices in the U.S., including early examples of successes and failures using donor oocytes to conceive. Through literature review and my own experience in the clinic, I describe how oocyte donors are screened and profiled prior to donation and provide a personal anecdote on the oocyte donation process as it occurred during my time at the clinic. Profiling of oocyte donors resembles the process of profiling sperm donors. However, oocyte donation is even more selective, and presents even greater ethical dilemmas—*what makes a quality*

donor? Who should and shouldn't donate oocytes? Oocyte donation is also a much more involved process than sperm donation. There are much more potential health risks associated with the oocyte donation process, so *how do we compensate women for their time and the risk associated with oocyte donation, without encouraging oocyte donation for income rather than altruism?* Compensation has been and continues to be a significant challenge behind the ethics of oocyte donation. I provide key examples of how price-fixing of oocyte donation has become both an ethical and legal matter and conclude with my personal thoughts on how the regulatory aspect of oocyte donation should be reworked for the benefit and protection of donors.

Development of Oocyte Donation in Fertility Medicine

The first reported pregnancy using donated oocytes was reported by Monash University's Department of Obstetrics and Gynecology in 1983 (Trounson et al., 1983). In Trounson et al.'s study, a 42-year-old female patient was undergoing an IVF cycle to generate embryos for her and her husband. The patient had five oocytes retrieved, kept four for her and her husband to use, and donated the last oocyte to a 38-year-old recipient who also struggled with infertility (Trounson et al., 1983). The recipient requested that the donated oocyte be inseminated using donor sperm. Roughly 12 hours after insemination, the recipient had a single embryo transfer and had a positive pregnancy test two weeks later (Trounson et al., 1983). Unfortunately, the patient experienced a miscarriage at 10 weeks' gestation (Trounson et al., 1983). Trounson et al. attributed this to the increased age of the donor but stated that the technique still showed promise and was the first successful transfer using an embryo created using donated oocytes.

Since 1983 over 50,000 births have occurred as a result of oocyte donation in the

United States, and each year, cycles using donor oocyte cycles account for over 10% of all IVF cycles conducted (Lindheim & Klock, 2018). Use of donor oocytes allows couples to choose oocytes from a donor that most closely resembles the mother and to use sperm from the male partner, so the child is still genetically related to one partner. On top of the costs associated with using ARTs to conceive, the addition of using donor oocytes for a patient's cycle raises the cost even higher. Couples conceiving using donor oocytes can expect to pay around $38,000 on average (CNY Fertility, 2020c). This value can vary significantly from bank to bank because many different factors influence cost: type of donor, medications, use of donor sperm if needed, embryo transfer, and short/long-term storage of embryos. Some clinics offer shared oocyte donor programs; women currently undergoing IVF for their own reproductive purposes donate half of their retrieved oocytes to another woman (Oppenheimer et al., 2018). In return, the recipient of the shared oocytes offers financial assistance to help pay for the donor's IVF cycle costs (Oppenheimer et al., 2018). Overall, costs are reduced for both parties involved, but shared donation is relatively unreliable in practice. If the patient undergoing the cycle decides to keep her oocytes or does not have enough oocytes retrieved to reasonably share half of them, this can dismantle the initial plan of sharing oocytes. For these reasons, shared oocyte donations are infrequent within fertility clinics.

Outside of the infrequent instances of shared donation, most oocytes are donated through independent cycles coordinated with egg banks. Oocyte donors go through ovarian stimulation for the sole purpose of donating those oocytes to someone else. There are three other kinds of oocyte donors: designated/known donors, semi-anonymous donors, and anonymous donors (CNY Fertility, 2020c). Designated or "known" donors

are women who are donating specifically to another couple—most often a family friend or relative. Semi-anonymous donors are equivalent to identity-release donors, who have consented to have their identity released to the parents and/or offspring later on in the child's life (CNY Fertility, 2020c). The majority of women who donate oocytes fall into the final category: anonymous donors. Anonymous donors relinquish all rights to any children created using their oocytes, similar to sperm donation conducted at a sperm bank. Anonymous oocyte donors wish to remain unknown to both the parents and any children conceived using their eggs—although personal genetic testing has compromised modern capabilities of remaining truly anonymous.

Donor Screening and Retrieval Process

The protocol for donating oocytes begins with extensive screening. Prospective oocyte donors meet with staff from the oocyte bank (more often called an "egg bank") for a consultation. Potential oocyte donors provide information about their family history, personal health history, lifestyle, and mental health. Oocyte donation programs have rigid requirements for donor age and will only accept donors who are between the ages of 21-29. Egg banks also require their donors to have a healthy weight—as underweight and overweight women respond to fertility medications differently and may have suboptimal egg quality (Bhattacharya et al., 2010).

After completion of the initial screening, donors have bloodwork and ultrasounds completed to ensure they are physically capable of undergoing the oocyte donation process and possess no underlying genetic conditions (California IVF Fertility Center, 2019). Once donors are medically cleared, donors meet with the egg banking facility's donor coordinator to discuss the next steps of the donation process. If the donor is

undergoing a cycle for a specific patient, there may be a donor contract drafted by an attorney for both the donor and potential recipients, intended to protect both parties (Falletta & Klein, 2021). The next step of the donation process is medicated ovulation induction. Most oocyte donation banks partner with local fertility clinics that control the donor's stimulation, cycle monitoring, retrieval and oocyte freezing process (California IVF Fertility Center, 2019). The bank is merely the facility that stores donated frozen eggs and handles clients looking to purchase donor oocytes.

During my time at the clinic, several women went through the donation process and had oocytes stored on-site for other patients. Our clinic referred to these women as "Donor egg vit" patients, meaning donor oocyte vitrification. Most donors at the clinic were anonymous, so we assigned an identification number to their profile, and all their oocytes were stored using that specific donor ID. In doing this, the embryology laboratory was able to keep track of the oocytes from each individual donor. This ensured that oocytes that were used, discarded, or purchased by another couple were documented without any risk of disclosing the donor identity to the patient unintentionally.

The stimulation protocol for oocyte donation mirrors the stimulation protocols for IVF: suppression, stimulation, and trigger. When the donor's follicles have reached the appropriate size during the stimulation phase, our physician instructs the donor to administer her trigger shot that evening and return to the clinic in 36 hours for her oocyte retrieval procedure. After the donor's retrieval is complete, the embryology lab counts the oocytes and reports the count back to the physician. All donor oocytes at the clinic are stripped of their cumulus cells prior to being frozen. We froze donor oocytes both individually and in pairs (depending on the number of oocytes retrieved) at the tip of a

vitrification device. Many kinds of vitrification devices are used in embryology labs, but we used Cryotop cryopreservation straws labeled with the donor ID and donor's date of birth. Since most donor oocytes will be frozen months or even years before a patient purchases them, we stored donor oocytes in our large cryochamber.

Compensation of Oocyte Donors

Since the inception of egg donation, compensation for donated oocytes has been widely contested worldwide. The word "donation" implies an altruistic gift with no reimbursement or reward. However, oocyte donation requires a significant amount of the donor's time for screening, monitoring, and the oocyte retrieval procedure, and comes with substantial medical risk for the donor. For these reasons, the ASRM decided that compensation of oocyte donors was ethically justified (Daar et al., 2016). In 1993, the initial payment structure proposed for donation of sperm and oocytes suggested an equal amount of pay for time spent on the donation process (Seibel & Kiessling, 1993). Seibel & Kiessling stated that sperm donors spend roughly one hour of their time on the entire donation process, versus roughly 56 hours for an oocyte donor. At the time, if a male patient received $25 for a single sperm sample that took roughly one hour of the patient's time to collect, this translated to $1,400 of compensation for an oocyte donor (Seibel & Kiessling, 1993). This figure was reevaluated in 2000 when compensation for sperm donors had reached $60-$70 per sample, which subsequently raised the anticipated compensation for oocyte donors to $3,360-$4,200 (Daar et al., 2016). This preliminary payment scheme accounted for differences in the amount of time required for sperm and oocyte donation. However, what this estimate did not take into consideration are the significant risks associated with oocyte donation that do not exist for donation of sperm.

As with women undergoing IVF, oocyte donors are at risk of developing ovarian hyperstimulation syndrome, infection at the site where stimulation medications are injected, or experiencing internal bleeding after the oocyte retrieval (Rizk & Smitz, 1992). Considering the potential side effects of controlled ovarian stimulation, the ASRM ethics committee constructed new guidelines in 2000 for oocyte donors. These guidelines encourage reasonable compensation for donors to acknowledge both the time and risk associated with donation (Daar et al., 2016). Amount of remuneration should not be related to the quality of the donor, number of oocytes donated, or quality of oocytes. The guidelines in 2000 also stated that clinics and egg banks that compensate a donor $5,000 or more for a single cycle needed to be able to provide justification, and that no donor should be paid more than $10,000 for any given cycle (Daar et al., 2016). Facilities affiliated with ASRM or SART were expected to abide by the guidelines or risk losing their membership with either society—which would lead to the facility being removed from approved donor agency listings.

Illegal Price Fixing of Oocyte Donor Compensation

Despite increasing demand for oocyte donors over time, the compensation guidelines established in 2000 by the ASRM remained unchallenged for over a decade. In 2011 a lawsuit was filed against the ASRM and SART by an egg donor who accused the societies of illegal price-fixing (*Kamakahi, et al. v. American Society for Reproductive Medicine*, 2011). By limiting compensation of oocyte donors to $5,000, fewer women are inclined to donate when compensation rates reflect oocyte donation practices from ten years prior when the demand for donated oocytes was lower (Daar et al., 2016). If less women are donating oocytes, there will be a decrease in supply, and potential clients will

have fewer donors to choose from. With a shortage in available donor oocytes, banking facilities can charge extravagant prices for the oocytes they do have available—to a point where some patients may be unable to afford them at all. Yet the donors do not benefit from this scarcity.

Lower compensation rates can also further divide oocyte donors by their education and economic status. Most egg banking facilities do not have the same educational requirements that sperm banks have for their donors. Using a basic, unfiltered donor search on The World Egg Bank website, I found that many of their oocyte donors have a high school diploma or some college experience but have yet to complete a degree (The World Egg Bank, 2020). By comparison, there are very few oocyte donors who have completed a bachelor's degree or graduate-level education (The World Egg Bank, 2020). This trend was also true for Donor Egg Bank USA, where many available egg donors have a high school diploma, some college, or have completed trade school (Donor Egg Bank USA, 2021). The negative relationship between oocyte donation and education level has been connected to income threshold (Krawiec, 2014). The U.S. Bureau of Labor Statistics reported in 2020 that the median weekly earnings for workers with only a high school diploma was $746/week and workers with "some college" earned $833/week. Workers who had a bachelor's degree earned $1,248/week, and post-graduate weekly earnings ranged from $1,497-$1,883 (U.S. Bureau of Labor Statistics, 2020). Individuals with higher socioeconomic status are arguably in a better position to weigh the pros and cons of oocyte donation (Krawiec, 2014). Women with higher education levels and higher income may refrain from donating at all, as the reimbursement amount for time and effort involved in oocyte donation is not sufficiently attractive. Without an increase

in compensation, this will further exacerbate the discrepancies seen in socioeconomic status of oocyte donors and may engender exploitation of women of lower economic status.

The guidelines put in place by the ASRM and SART have served as guidelines for many years, with few sanctions for the clinics and sperm/oocyte banks who do not choose to follow them. Despite the arguments made during the *Kamakahi, et al. v. American Society for Reproductive Medicine* case, the ASRM still stood behind their initial set of guidelines, as the intent of the guidelines was to prevent commodification of oocytes. However, the settlement ultimately ended in 2016 with the ASRM revoking their outdated guidelines. This allowed facilities to decide their own payment structure for oocyte donation.

A Growing Oocyte Economy

Following withdrawal of the ASRM/SART guidelines for oocyte compensation, oocyte donation has evolved into a marketplace shaped by consumer behavior. Many clinics and egg banking facilities have increased their reimbursement plans to compensate donors more than $5,000 per cycle to attract more donors to their programs. Compensation for oocyte donors contracted through traditional egg banking facilities ranges from around $3,500-$30,000 per cycle (Egg Donation Inc., 2020). First-time oocyte donors are compensated less, but after a successful cycle, banks encourage donors to donate multiple times with the incentive of higher compensation for each subsequent donation (Egg Donation Inc., 2020). Infertile women have also begun to seek private donors through forums, social media, and online advertising to find a donor to provide oocytes for them. Patients desperate to become pregnant are willing to pay tens of

thousands of dollars for a direct donor. Each of these practices is problematic, as they incentivize exploitation of women for their oocytes. Oocyte donation is not intended to be a primary form of income for any woman, and current legislation does not protect or prevent oocyte donors from undergoing repeated cycles for higher incentives. The ASRM/SART does recommend limiting donations to six cycles or fewer (PC-ASRM/SART, 2020).

This newly formed oocyte economy is an uncharted territory that epitomizes the "Wild West" narrative of the fertility industry described by Marcy Darnovsky of the Center for Genetics and Society (Ollove, 2015). There is an urgent need for development of present-day regulation of oocyte donation by the ASRM and SART in order to protect oocyte donors from exploitation. I agree with compensating donors for time and risk associated with oocyte donation rather than quality or number of oocytes. However, “time” and “risk” are relative terms, and the definition varies between donors, intended parents, and clinics. Time and risk also have different definitions to lawyers and insurance companies as well. The previous “guidelines” made by these self-regulated entities have proven to be insufficient, which necessitates increased regulatory oversight to protect the well-being of donors, intending parents, and children conceived through donor gametes.

Pre-Implantation Genetic Diagnostics and Testing

Since the inception of ARTs, physicians and scientists have revised protocols and techniques used for IVF to provide the best possible care to patients. Existing protocols may require modifications when new information is shared within the medical community, or when new technologies become available. The continual flow of new and

improved information into the field of fertility medicine has led to rapid advancements previously not considered possible. One of the newest techniques attributed to increased success of IVF cycles is the implementation of genetic testing. Genetic testing, also called "genetic screening" is used to detect variations present in an individual's genetic makeup. Some genetic variations may be insignificant, while others can cause heritable diseases and disorders that affect an individual throughout their lifetime.

In this section, I describe the history of genetic screening in reproductive medicine, starting with carrier screening performed through amniocentesis and chorionic villus sampling in maternal-fetal medicine, leading to the development of pre-implantation genetic diagnostics performed on embryos in the laboratory. The term pre-implantation genetic diagnostics has been re-named "pre-implantation genetic testing" [PGT], then broken into smaller categories based on what each test is screening for, which I describe in detail. During my time at the clinic, I began to learn embryo biopsy technique, and how to interpret the results of genetic testing we received. I provide my personal account of "The PGT Process" as it occurred in our laboratory, before exploring why PGT has become ethically controversial issue. To understand the opinions of "key players" in the fertility field, I examine public, clinical, and laboratory opinions about PGT through review of interviews and statements given by each group. To conclude, I discuss the goals and future implications of PGT in reproductive medicine.

Our understanding of human genetics has swiftly evolved over the past 60 years. The structure of DNA was first described as a double helix in 1953 (Watson & Crick, 1953). This monumental discovery initiated a cascade of studies on the human genome. In 1966, Tjio and Levan correctly identified the number of human chromosomes as 46,

which allowed researchers to study the structure and function of human chromosomes more closely (Tjio & Levan, 1966). From this point forward, scientists began to use human genes as a diagnostic tool. Abnormally shaped, missing, or extra chromosomes are associated with specific disorders such as Turner and Klinefelter syndrome, Down syndrome and XXY disorder. As additional disorders were characterized, a deeper understanding of how genes were organized on chromosomes led to more detailed diagnostic capabilities. A patient's own genetic information could be used to determine the likelihood that their child would inherit a disease or disorder from them, called *carrier screening*.

Genetic screening practices were also implemented early on in maternal-fetal medicine in the 1960's with the development of amniocentesis (Levy & Stosic, 2019). Amniocentesis is an invasive procedure that withdraws a sample of amniotic fluid from the womb during pregnancy which is used for genetic screening (Levy & Stosic, 2019). Within the same timeframe, an equally invasive alternative to amniocentesis was made available: chorionic villus sampling. Chorionic villus sampling, or CVS uses a thin needle to remove cells from the placenta for genetic testing (Alfirevic et al., 2017). Despite the invasive protocol, CVS was the most accurate method of fetal genetic screening throughout the 1980's and 1990's and could be performed up to six weeks earlier than amniocentesis (Levy & Stosic, 2019).

However, with IVF becoming a more prevalent technique in reproductive medicine, there was an unfilled need for genetic screening before the fetal stage. In 1990, Handyside et al. reported the first application of genetic screening in embryos. Two couples at risk for transmission of X-linked genetic disorders volunteered to have their

embryos analyzed using a new technique: Pre-Implantation Genetic Diagnostics [PGD] (Handyside et al., 1990). The initial intent of PGD was to screen embryos for debilitating sex-linked disorders. This screening technique ultimately improved pregnancy outcomes for these patients, as embryos with genetic abnormalities tend to have a lower chance of successful implantation (El-Toukhy et al., 2008). Advancements in screening techniques and diagnostic potential diversified the application of PGD, and today term "PGD" is considered outdated. Clinics now refer to screening of embryos for genetic abnormalities as PGT or Pre-Implantation Genetic Testing. There are three different types of PGT that examine embryos for different disorders (outlined in Table 8). Patients can select embryos based on a combination of morphology grading and genetic screening, giving them the best possible chance at conception on their first embryo transfer. Throughout this section, the term "PGT" will be used to encompass all three types of PGT testing: PGT-A, PGT-M and PGT-SR.

The PGT Process

There are three major steps of PGT: what is called assisted hatching, biopsy, and screening. As an embryology trainee, I began performing these steps on embryos under the supervision of senior embryology staff. In most cases, a patient donated some oocytes during their IVF cycle and designated them for research purposes. I inseminated those donated oocytes using the patient's partner's sperm (or donor sperm sample) and cultured the fertilized oocytes into blastocysts. At this point, I could begin the steps involved for carrying out PGT. The process of preparing for PGT begins on day three with laser-assisted hatching (LAH). Laser-assisted hatching is a procedure that utilizes a quick pulse of an infrared laser to bore a hole in the outer shell of the embryo, also called the zona

pellucida (Hammadeh et al., 2011). The hole in the zona facilitates embryo hatching—a step that is crucial for successful implantation, but it also useful for embryo biopsy to perform PGT. Embryo biopsy can be performed on hatched embryos, or non-hatched embryos, but our lab always used LAH to manually facilitate hatching which made aspiration of the trophectoderm easier (Aoyama & Kato, 2020). Performing LAH was a relatively quick process. I used the laser to make one to three pulses on the zona of each embryo, enough to create a small pinhole in the zona. After completing the assisted hatching process, I would cover the dish of embryos and return the dish to the temperature-controlled, humidified incubator. Over the next several days, the embryo would begin to "hatch out" through the hole that I made in the zona.

Five days post-fertilization, the cells within the embryo have divided several times and have begun to form two distinctly different cell lines in what is referred to as a blastocyst. The inner cellular mass (ICM/embryoblast) are the cells that will become the fetus, and the Trophectoderm Epithelium (TE/trophoblast) cells become the placenta (Wolpert, 2007). Blastocysts that have hatched successfully will have cells from the trophectoderm present outside of the zona, with cells of the embryoblast still housed within the zona. Day five blastocysts are preferred for PGT because they are more resilient to the biopsy procedure than earlier-stage blastocysts (Scott et al., 2013). It has also been reported that embryos with higher degrees of mosaicism, or mixed normal/abnormal cells are more likely to self-correct if PGT is conducted on day three rather than day five (Barbash-Hazan et al., 2009). The term "self-correct" refers to an embryo's ability to expel debris and cellular fragments (Orvieto et al., 2020). Cellular debris and fragmentation within an embryo are elements that would make an embryo

"lower quality" in the eyes of an embryologist. For these reasons, it is preferential to conduct genetic testing on blastocysts that have had more time to grow and begin to compartmentalize before removing cells for testing. An example of a blastocyst hatching out from the zona is demonstrated in Figure 14. In this photograph, the trophectoderm has hatched out, but the embryoblast is still encapsulated by the zona.

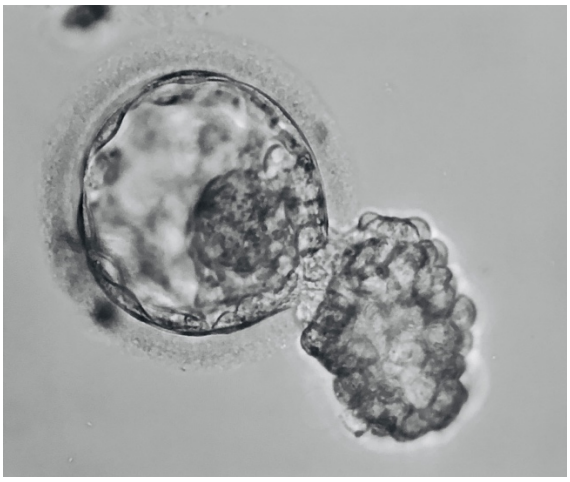

Figure 14. Embryo Post-Laser-Assisted Hatching. This is a photo of a blastocyst that I had performed laser-assisted hatching on several days prior. The trophectoderm cells are "hatching" out of the embryo. Photo Credit: Shelbi Peck, 2019.

Next, the biopsy process can begin. First, I moved each blastocyst into its own individual micro-drop of nutrient-rich media in a shallow dish. This step is crucial for ensuring that the biopsied clump of cells stays with the corresponding blastocyst. Having more than one blastocyst in a single drop could lead to confusion regarding which biopsy was taken from which blastocyst when the biopsies are being sent off for testing. If biopsies and embryos are mixed up, this could lead to the lab inaccurately diagnosing

embryos as genetically abnormal when they may actually be normal, or vice versa. After I moved each blastocyst into its own micro drop in the dish, the embryos are returned to the incubator while the microscope is set up for biopsy. The microinjectors we used for embryo biopsy are the same microinjectors we used to perform embryo biopsies. My preferred setup was to have the holding needle on my left, which holds the blastocyst in place during biopsy, and the blunt biopsy needle on my right that is pre-loaded with a thick oil. This oil gave me better control with the needle when I aspirated a clump of cells off the trophectoderm during biopsy. I used the blunt needle to pull the trophectoderm cells taught and used several pulses of the non-contact laser to separate off a small clump of the cells. Once the biopsied cells separate, I placed the biopsy next to the blastocyst in the drop and repeated the process for the other blastocysts. After I completed all the biopsies, I numbered each embryo and biopsy for our records and for the screening facility, and individually loaded the biopsies into labeled tubes before sending them out for genetic testing.

PGT is typically performed through Polymerase Chain Reaction (PCR), or Fluorescence In-Situ Hybridization (FISH) (Fasouliotis et al., 1998). PCR amplifies the DNA from the blastocyst so the genetic code can be read, and genetic disorders can be detected (McArthur et al., 2005). FISH utilizes small fluorescent probes that adhere to translocation sites on chromosomes, or places where chromosomes have broken apart and bound to other chromosomes (McArthur et al., 2005). Depending on which PGT test is being used, one or both methods may be performed. Once we receive the testing results for the biopsies, we upload the patient's PGT Report on our Electronic Health Record system, where the patient can see each of their blastocyst's results. The appearance of the

PGT report varies depending on which company performed the screening. However, the information the report provides is the same. Each blastocyst is given a normal or abnormal result with a list of the genetic abnormalities present, if any. At our clinic, we most sent embryo biopsies for PGT to Invitae or CooperGenomics for screening, as opposed to screening embryos in-house. Once we uploaded the patient's results to their profile, our physician would contact the patient to help them understand their PGT report. The patients then decide if they would like to keep or discard certain blastocysts based upon the results of their testing.

A Controversial Technique with a Contentious Future

Applications of PGT in reproductive medicine have been met with mixed reactions. PGT uses well-established protocols to help patients with reduced fertility to decide which embryos have the best possible chance at implanting and becoming a healthy child—the fundamental purpose of IVF and ultimate goal of patients that seek this service (McArthur et al., 2005). One of the greatest concerns about PGT is the invasiveness of the entire process—particularly the use of the infrared laser during hatching, pulling on the hatching trophectoderm, and use of the laser again to dislodge a clump of cells for testing. The intensity of the PGT procedure has raised concerns that this technique may have negative impacts on growth and development later in life. While these assumptions have not been proven, PGT is still a relatively new technique and there is a lack of long-term studies available that examine the potential negative impacts related to the procedure that may occur later in life.

In addition, numerous ethical issues surround the use of PGT in reproductive medicine. The initial debate among ethicists, scientists, and physicians about PGT was

whether discarding a genetically abnormal embryo constituted an abortion. Depending on how an individual defines the start of life, some argued that the termination of life could also include the disposition of an embryo (Cameron & Williamson, 2003). Debate over the moral status of embryos ensued at reproductive medicine conferences around the world, but it was ultimately determined that discarding embryos (normal and abnormal) is not a form of abortion. In more recent years, use of PGT is being challenged once more, as scientists and ethicists grapple with the potential implementation of genome-editing technologies into embryology. Genome-editing technologies present a new way to "edit" sequences of DNA using specialized proteins. There are several different methods for genome-editing: Zinc-finger nucleases, transcription activator-like effector nucleases, and the newest (and currently the most highly contested) method CRISPR/Cas9 (Cavaliere, 2017). The CRISPR method guides Cas9 proteins to specific sites on a strand of DNA and cuts the strand. The open strand of DNA can then be altered, and new or modified genes can be inserted at that site (Cavaliere, 2017).

This technology could have a profound impact on reproductive medicine, as genetic disorders could essentially be "cut out" or modified out of an embryo's genome. CRISPR could arguably be considered therapeutic because it can treat embryo for a genetic disorder; however, this could very well lead to patients wanting to select embryos for non-medical purposes—such as selecting desirable physical traits, and genetically modifying their embryos to meet certain standards. This deviates from the intended use of PGT which solely provides information on an embryo based on its genetic quality (Cavaliere, 2017). Research conducted on the use of CRISPR in fertility is still in its infancy but has received intense criticism from ethicists and the public alike. Studies

using CRISPR on human embryos will likely be limited, as the NIH released a statement in 2015 disapproving of the use of genome-editing technologies on human embryos and stated they will not fund projects that propose this kind of work (Collins, 2015).

Opinions on PGT

The entire PGT process requires significant physical and emotional contributions from multiple parties: the physician, scientist, and patient. The physician contributes a comprehensive treatment plan, provides counseling to patients, and performs the oocyte retrieval procedure. Laboratory scientists provide technical expertise for culturing oocytes and embryos, fertilization in vitro, and performing the biopsy procedure. Finally, the patient's (both partners) contributions include time, a significant emotional/financial investment, and the physical investment of their oocytes and sperm. If one role is not fulfilled or is performed improperly, this could put the patient's entire cycle at risk of failure. Due to these different roles, rights and responsibilities of each group, physicians, scientists, and patients each have their own opinions about who should use PGT, how the process is carried out, if there should be limits on the use of PGT, and if so, what those limits should be.

Doctors have long been held to four foundational ethical principles of care: autonomy, beneficence, nonmaleficence, and justice (Beauchamp & Childress, 2001). These standards are designed to instruct physicians in making the most ethical and responsible decisions for patients while practicing medicine and are vital in decision-making for PGT. Obstetricians and Gynecologists (OGBYN's) tend to be the most well-informed physicians regarding the purpose, benefits, and risks of PGT. Physicians seeing patients suffering from infertility view PGT as a method for understanding the underlying

causes of infertility and unsuccessful embryo transfers. Dr. Cynthia Austin, Director of Cleveland Clinic's IVF program stated, "For someone who has had to face the agonizing decision to abort a previous pregnancy due to a problem, or faced a loss due to one, the decision is clear." (Cleveland Clinic, 2017). To OBGYN's, the resounding opinion on PGT is that patient autonomy outweighs the physician's personal opinion of genetic testing of embryos. Properly informing the patient about the risks, benefits, results, and options for genetic testing is crucial, and patients can make informed decisions to pursue or abstain from PGT on their embryos (Cleveland Clinic, 2017). Discarding genetically abnormal embryos is also seen as a more ethical decision for both the patient and physician than aborting a genetically abnormal fetus, but either way, the decision should always the up to the patient (Cleveland Clinic, 2017).

Laboratory scientists, particularly embryologists, have fewer interactions with patients but provide technical expertise and the physical act of performing embryo biopsy for PGT. With advancements in culturing and biopsy of embryos, PGT is a relatively safe and effective procedure (Maxwell & Grifo, 2018). In a study conducted by the Society for Assisted Reproductive Technology (SART), implantation and delivery rates were higher for patients who chose to have PGT conducted on their embryos and experienced fewer miscarriages. Their dataset from 2016 also demonstrated that PGT was a very effective tool for predicting pregnancy outcomes in women over the age of 38 (SART, 2018). Yury Verlinsky, former director of the Reproductive Genetics Institute in Chicago also acknowledged PGT as a beneficial technique in IVF for multiple reasons: PGT expedites successful conception, as implantation rates double for genetically normal embryos. Secondly, PGT can reduce the risk of multiple births in IVF if the embryo's

genetic status is known prior to transfer (Hunter, 2004). For many scientists, the benefits of PGT outweigh the clinical risks and is in the best interest of both patients and physicians, as it increases the likelihood of successful implantation and delivery and reduces miscarriage rates (Maxwell & Grifo, 2018). Where the benefits and ethical justification of PGT stop, however, is when it is being used for societal engineering, such as the selection of physical traits rather than to prevent inheriting medical conditions.

The party that is ultimately affected by PGT the most are the patients who use it. Patients deserve to have full reproductive autonomy. They should also be properly informed of the benefits of PGT, associated risks, and be provided counsel on how to cope with unfavorable results. If patients feel like they have more control to make informed decisions, PGT has received favorable reviews from patients (Järvholm et al., 2017). In a three-year follow up interview with 22 different couples, many discussed how PGT has allowed them to have early insight into their chances of a conceiving a healthy child and was a better option than aborting a genetically abnormal fetus mid-pregnancy. One participant commented "It was the best decision we have ever made; I think. Now we know. Our child is healthy… and if he wants to have children, he doesn't have to think about this." (Järvholm et al., 2017).

Use of PGT enables couples to make informed decisions regarding which embryo they should transfer and empowers them because they know that their child will be born without any genetic disorders. Although not all patients receive encouraging results from PGT, couples with all abnormal embryos that could not undergo transfer still saw value in PGT. One couple remarked that "—you seldom regret something that you do, more likely the things that you don't do" (Järvholm et al., 2017). Patients understood that

genetic testing never guaranteed normal embryos but did prevent false hope of transferring embryos with unknown genetic status, which decreases the likelihood of experiencing a miscarriage later on.

Goals and Implications of PGT

Use of PGT in reproductive medicine allows for greater patient autonomy, as patients can use their results to make more informed decisions in their family planning. Sometimes patients will choose not to have PGT performed on their embryos initially because the idea of having six embryos of unknown genetic quality seems more promising than knowing that some (or all) of their embryos are genetically abnormal. This can create a false sense of hope and lead to failed transfers, but patients can have their remaining embryos biopsied for PGT at any time. At the clinic, some of our patients chose not to have PGT performed on their embryos, but after having a failed embryo transfer, decided to have their remaining embryos tested to see if they have any embryos that are genetically normal. Patients also have a choice of which company performs and reports their PGT results to them and can change companies for different cycles if preferred. Educated medical autonomy should always be paramount, including the pursuit of genetic testing of a patient's own embryos.

For now, the overarching goal of PGT remains to improve IVF cycle outcomes for patients, namely through avoiding transfer of genetically abnormal embryos that have a smaller chance of successful implantation (Maxwell & Grifo, 2018). With the international interest of incorporating gene-editing technologies into human medicine, if left unregulated, PGT could eventually develop into a method for non-medical embryo selection, a purpose it was not initially intended for. Going forward, gene editing

technologies and PGT should remain separate entities, and legislators should anticipate that certain groups may want to take advantage of the current gray area, especially outside of the U.S. Technologies introduced into the field of fertility medicine have changed the way infertility is diagnosed and treated, which has benefitted couples across the globe and led to the births of millions of babies over time. PGT in its current form has improved cycle outcomes while posing minimal risk to embryonic development. However, there is an urgent need for studies and firm legislation regarding future implications of genetic testing. Primarily to prevent PGT from being used as a step toward genetic manipulation as gene editing technologies become more available in medical research.

Note: Portions of section 5.5 were submitted for credit as a part of BIO516: Foundations of Bioethics, with permission to utilize in this dissertation from Dr. Jason Scott Robert.

Conclusion

Many techniques used in fertility have problematic beginnings and even more precarious implications going forward. The ethical issues surrounding sperm and oocyte donor profiling/anonymity, compensation for oocyte donors, and the uses and implications of genetic testing became very apparent to me once I played a more active role in the fertility field. The capacity to control human reproduction has far surpassed the current regulations in place that protect patients and the children born using ARTs. Resolving ethical problems surrounding some of the questionable practices in fertility medicine will require adopting new policies, imposing restrictions, and enforcing sanctions upon clinics who do not comply. I believe that in a field like fertility that is currently so underregulated, effective legislation will need to be put in place at the federal

and state level and be fully endorsed by the SART and ASRM. Closing the loopholes that have allowed for ethical issues to arise, and having coherent legislature will provide the clarity, consistency, and accountability that the field needs to more effectively protect all parties involved in reproductive medicine.

CHAPTER 6

CURRENT AND FUTURE RESEARCH AIMED AT IMPROVING FERTILITY MEDICINE AND ARTS

Synopsis

Reproductive medicine is a complex, specialized field with two overarching goals: to treat patients experiencing reproductive dysfunction, and to improve upon existing diagnostic methods and treatment options for patients. To accomplish these goals, research is needed to broaden our knowledge of reproduction. Research lays the groundwork for the development of new technologies, better diagnostic capabilities, and ultimately improvement in the scope and quality of care provided to patients. In this chapter, I describe several areas of research that are in the early stages of clinical implementation aimed to address sources of infertility. This includes uterine transplants, ovarian tissue cryopreservation, and FSH therapy for spermatogenesis. Then, moving into the IVF laboratory, I discuss artificial intelligence as a mechanism for improving oocyte and embryo culture, and give a personal account as to how these technologies would be implemented and beneficial at the fertility clinic where I worked. Finally, I discuss modern fertility testing methods for men and women as a form of remote fertility testing.

Contributions and Categories of Research

Over time, studies within the field of reproductive medicine have deepened our understanding of the intricate relationship between the anatomical and physiological mechanisms underlying conception and gestation. This has led to new diagnoses, more efficient stim protocols, improved quality of care, and new advanced reproductive technologies available to help patients conceive. Conditions such as endometriosis,

primary ovarian insufficiency, azoospermia and PCOS are relatively common, well-studied reproductive disorders that can be treated or managed using current fertility treatments and Assisted Reproductive Technologies [ARTs]. However, the idiopathic or "unexplained" infertility is still a prevalent diagnosis in fertility medicine that accounts for roughly one-third of all cases of infertility (Chandra et. al, 2013). Diagnosing a patient's source of infertility can be challenging for even the most experienced physician due to the complexity of human biology. Infertility can manifest as a single-factor disorder, but more often exists as a multi-factorial condition where generic fertility testing may be insufficient to definitively diagnose.

Fertility treatment plans are individually curated for each patient to encourage the best results while minimizing the likelihood of adverse side effects. An effective and ethical fertility treatment plan also takes into consideration the patient's physical wellbeing, emotional health, and financial limitations. Even for patients experiencing a known, well-studied cause of reduced fertility, trying to conceive can be a frustrating, costly, and emotionally draining journey and the many events leading up to conception require a significant commitment of both money and time. Frequent trips to the clinic for ultrasounds and blood draws, daily injections, and the procedure itself (IUI, IVF) take weeks of preparation. This process is further exacerbated for patients with no clear reason for infertility where typical stimulation protocols and treatment options may have lower success rates and require several cycles before a pregnancy is achieved. Without a clear diagnosis, the "treatment plan" for unknown infertility is not as clear-cut and becomes a process of trial and error until the patient has a successful cycle.

Research conducted in the field of reproductive medicine can lead to advancements that change the way we treat patients experiencing known infertility disorders, uncover additional causes of reduced fertility, enhance current ART protocols, or improve future ART cycle outcomes. The process of implementing new methods for overcoming reduced fertility takes years of pre-clinical studies, clinical trials, and long-term studies before physicians can incorporate a new protocol or ART into their practice. Most studies conducted on reproduction and fertility for both men and women fall into one of three broad categories: infertility diagnostics, treatments, and fertility preservation. Infertility diagnostics include the improvement of current diagnostic techniques as well as the development of new ways to diagnose infertility. Infertility treatments encompass the development of new surgical procedures, bioengineering techniques and new medications or therapies aimed to treat cases of known or idiopathic infertility.

The final category of fertility research is fertility preservation—a process by which oocytes and sperm are cryopreserved outside of the body for future use. Fertility preservation was initially developed for patients undergoing radiation or chemotherapy for cancer treatment. These treatments destroy malignant cancer cells in the body through targeted beams of high-energy or potent chemotherapeutic drugs. However, radiation and chemotherapy can wreak havoc on the reproductive system, rendering a patient infertile or with severely damaged reproductive organs. When a patient receives a cancer diagnosis, especially for cancer located within or near the reproductive system, they are often encouraged to preserve sperm or oocytes prior to undergoing treatment. Through fertility preservation, patients can preserve their capacity to have biological children even if chemotherapy or radiation destroys their natural ability to conceive. In more recent

years, fertility preservation has taken on a contemporary meaning for women as a tool to circumvent the adverse effect that increasing maternal age has on fertility. Oocytes are frozen in their current state, halting their natural decline in quality over time. Using cryopreservation extends the number of years that a woman can conceive, giving her more reproductive freedom through the nullification of the "biological clock."

Uterine Transplantation

The uterus plays several different roles in the process of conception. In this section, I cover a new form of addressing severe uterine-factor infertility—the uterine transplant. First, I contextualize uterine-factor infertility by briefly describing the role of the uterus in conception and document the prevalence of uterine-factor infertility worldwide. Next, I discuss the process of uterine transplantation, noting early successes and failures of the procedure. Using a thorough literature review, I report on public opinions regarding uterine transplants as a form of fertility treatment and discuss the reproductive implications of using uterine transplantation as a temporary form of treatment in fertility medicine, using a personal account from my time at the clinic.

Small contractions facilitate movement of sperm up to the fallopian tube for fertilization of the ovulated oocyte (Betts et al., 2013). Following ovulation, a surge of progesterone produced by the ruptured ovarian follicle initiates proliferation of the uterine lining in preparation for implantation (Betts et al., 2013). The endometrial lining becomes thicker through a process called *proliferation*, or rapid division of cells (Brosens et al., 2002). As the lining thickens, it becomes vascularized with additional blood capillaries and tightly coiled uterine glands begin to elongate and release secretions of proteins and growth factors that help facilitate implantation (Brosens et al., 2002). If

implantation does not occur, the lining is shed as discharge and the uterus can begin to generate a new lining during the next cycle (Betts et al., 2013). If implantation does occur, the uterus plays the most significant and long-lasting role in human reproduction, providing structure and nutrients throughout roughly 40 weeks of fetal gestation, as well as strong contractions during labor and delivery.

Uterine factor infertility is a known contributor of increased time-to-pregnancy and a significant cause of recurrent pregnancy loss in women (Flyckt et al., 2017). Over 1.5 million women worldwide have been diagnosed with uterine factor infertility, either due to congenital absence of the uterus or having a non-functioning uterus (Flyckt et al., 2017). Conditions such as septate uterus and bicornate uterus may be surgically corrected through a procedure called metroplasty, which removes the tissue dividing the uterine body to make the uterus pear-shaped again (Haydardedeoğlu et al., 2018). Previous research has shown a positive correlation between the metroplasty procedure and pregnancy outcomes (Haydardedeoğlu et al., 2018). However, there are few effective treatment options for the numerous other causes of uterine factor infertility, and even fewer for women with absolute uterine factor infertility (Hur et al., 2019). Due to the long-term role of the uterus in gestation, infertility related to uterine dysfunction can be difficult to treat effectively. Compromised uterine health impacts conception. An absent or non-functional uterus reduces the efficacy of traditional less-invasive fertility treatments such as timed intercourse and IUIs. Even if conception is successful initially, uterine factor infertility negatively impacts the patient's ability to carry a pregnancy to full term. Recurrent pregnancy loss due to uterine dysfunction often requires that a patient use a gestational carrier to carry the pregnancy for them.

Use of a gestational carrier has shown to improve pregnancy outcomes for women with uterine-factor infertility (Murugappan et al., 2018). In order to have biological children through the use of a gestational carrier, patients first undergo an IVF cycle to generate their own embryos before having them transferred into the carrier who will carry the pregnancy and deliver the baby for them. While the use of a gestational carrier makes starting a family possible for these patients, it is not a medical solution for uterine factor infertility and comes at a substantial financial cost on top of the IVF cycle itself (CNY Fertility, 2020a). The cost of having another woman carry the pregnancy for a patient varies from agency to agency, but CNY Fertility estimates the cost of using a gestational carrier to be roughly $100,000 on top of the cost of the patient undergoing IVF (CNY Fertility, 2020a). Being a gestational carrier is not currently legal in all countries but is legal within the U.S. Some states allow compensation for gestational carriers, while others prohibit it, making the service purely altruistic (Hur et al., 2019). Regardless of the availability of gestational carriers in each state, some patients may object to using a carrier due to ethical or religious reasons.

For many years, undergoing IVF and using a gestational carrier was the only solution for patients with severe/absolute uterine factor infertility to have biological children. However, a promising new treatment has emerged for absolute uterine factor infertility: the uterine transplant [UTx] (Hur et al., 2019). The UTx procedure is the first proposed treatment for absolute uterine factor infertility, and the process closely resembles transplantation of other organs that are more routinely performed today (Brännström et al., 2015). Organs such as the heart, kidneys, lungs, liver, and pancreas have been transplanted from donors to recipients for decades (Cleveland Clinic, 2021).

Despite the numerous risks and side effects associated with organ transplantation, the benefits of receiving a vital organ transplant tend to outweigh the risks. Transplantation of a non-vital organ such as the uterus is regarded as a non-essential, elective surgery that is still highly experimental. To prepare for an organ transplant or UTx, the recipient must receive a comprehensive evaluation of their medical history and attend consultations with transplantation physicians, surgeons, pharmacists, and financial coordinators (HHS, 2019c). All consultations and exams are completed before the patient can be placed on the waiting list to find a blood-type match to a donor (HHS, 2019c).

A UTx can be performed using a living or deceased donor, similar to kidney or lung transplants (Hur et al., 2019). A living donor can still thrive without a uterus, with a single kidney or after donating a portion of their lung. Being able to accept living or deceased donor organs can shorten the amount of time that a patient spends on the waiting list to receive a transplant but finding a match can still take months or even years. Once a match is found, the patient is placed on immunosuppressant medications to weaken their immune system, which facilitates acceptance of the new organ in the recipient's body (Allison, 2016). Surgical procedures for both the donor and recipient are coordinated within just a few hours of one another so the organ spends as little time outside of the body as possible. Following transplantation of these vital organs, the patient typically remains on immunosuppressant medications for the remainder of their life to prevent rejection, toxicity, and potential infection of the organ (Ensor et al., 2011). Unlike transplantation of a vital organ, the UTx procedure is not intended to be permanent (Hur et al., 2019). Assuming there are no complications, the transplanted uterus could be removed through a hysterectomy procedure after the patient has no more

than two pregnancies resulting in a live birth (Hur et al., 2019). Once the transplanted uterus has been removed the patient can stop immunosuppressive therapy (Berkane et al., 2020).

The UTx has been anecdotally attempted several times throughout history but has lacked effective protocols, controls, and recordkeeping. The earliest recorded case of UTx was performed in Saudi Arabia in 2000 (Taneja et al., 2018). Saudi Arabian media shared a story of a 26-year-old patient who received a transplant from a living donor that resulted in a hysterectomy three months after due to uterine tissue decay (Nair et al., 2008). Due to the lack of success of the procedure, as well as the legal, ethical, and social pushback further study on the UTx procedure stopped for over a decade (Taneja et al., 2018). In 2011, a 22-year-old Turkish woman reportedly received a uterine transplant and experienced several miscarriages before undergoing an IVF cycle that led to a single birth in 2020 (Catsanos et al, 2013; Malasevskaia & Al-Awadhi, 2021). Although these cases were anecdotal and do not demonstrate thorough and established protocols, they do serve an important role in the history and development of the procedure itself. The first scientifically published study on uterine transfer was published in 2014 by Dr. Mats Brännström in Sweden (Taneja et al., 2018). Nine female patients received transplants, all of whom were experiencing uterine factor infertility due to congenital absence of the uterus or previous hysterectomy (Brännström et al., 2014). Transplant patients were approved to begin embryo transfers within six months if no postoperative complications occurred, and in this study only two patients experienced complications that required an emergency hysterectomy (Brännström et al., 2014). The remaining seven women experienced normal menstrual cycles post-transplant and underwent embryo transfers. In

2017, Brännström reported that six babies had been born between the seven women so far, with two additional ongoing pregnancies expected to deliver later in the year (Brännström, 2017).

A year later, Brännström and his team published a follow-up report for one of their previous UTx procedures involving the transfer from a 61-year-old donor to a 35-year-old patient (Brännström et al., 2015). The recipient underwent an embryo transfer after IVF and had a single birth in 2015 (Brännström et al., 2015). This was the first study that documented an actual live birth that resulted from a transplanted uterus. Following documented success of the procedure in Sweden, live births from UTx patients have been reported in the People's Republic of China, India, and the U.S. The first transplant of a uterus performed in the U.S. occurred at the Cleveland Clinic in 2016. Unfortunately, one-week post-transplant the 26-year-old patient developed a severe yeast infection that required a hysterectomy to remove the transplanted uterus (Flyckt et al., 2017). Despite overall failure of the procedure, this case was unique in that it was the first UTx to take place in the U.S., and the uterus was retrieved from a deceased donor rather than a living donor (Flyckt et al., 2017). While complications with the procedure may have been unique to the patient, the outcome of the Cleveland Clinic case revealed that there are still many unknowns regarding the success of UTx and highlighted one of the many potential side effects that immunosuppressant medications may have on the efficacy of transplantation (Flyckt et al., 2017).

Reports of the Cleveland Clinic UTx made headlines around the country, which led to the development of the important question: *should we be doing uterine transplants in the first place*? In 2017, results of a cross-sectional public survey addressed public

opinions and attitudes on the use of UTx as a means for addressing infertility in women. Over 78% of their 1,247 survey respondents stated that they believed transplantation of a uterus from one woman to another is ethical (Hariton et al, 2018). Only 4% of respondents opposed UTx, and the remainder of survey respondents were neutral regarding the ethicality of the procedure (Hariton et al, 2018). Hariton et al.'s study also found that support of the procedure was higher in female respondents, as well as those with higher yearly income and higher education levels. Alternatively, opposition towards the procedure was related to religion, cost, or indifference towards having biological children (Hariton et al, 2018).

Clinical trials for UTx's continued, and in 2020 the Baylor College of Medicine published a three-year study that tracked pregnancy outcomes of 20 women who had received a UTx from living donors. Prior to their transplant, all 20 patients had undergone an IVF cycle to generate viable embryos (Putman et al., 2020). Fourteen out of 20 patients had no postoperative complications and were able to begin embryo transfers (Putman et al., 2020). Following development and expansion of the embryonic cavity, good-quality embryos were used for transfers. Out of the 14 successful UTx procedures, 13 patients gave birth or became pregnant during the timeframe of the study (Putman et al., 2020).

The live births reported over the past five years using UTx are encouraging for both physicians and patients. Even if the procedure is used as a last resort option for the most severe cases of uterine factor infertility, UTx is still considered experimental at this point and is costly to perform. I nonetheless chose to discuss the development of UTx because I feel that the procedure presents a promising avenue for restoring fertility in

women with severe uterine dysfunction. During my time at the clinic, we would fill out a National Assisted Reproductive Technology Surveillance System [NASS] worksheet for each patient who went through an IVF cycle at the clinic. That information we filled out about each patient on the NASS worksheet was entered into the CDC's data collection site using a unique ID that was generated for each patient. If a patient went through an additional IVF cycle, her data would be reported to the NASS so all her information would be recorded in one place.

Basic information for our patients included demographics, obstetric history, prior treatment, and the patient's reason for infertility. We recorded information about their current cycle, specifically when they began taking stimulation medications and what type of stimulation protocol was used. The NASS also requires laboratory information for each patient. This included what type of IVF cycle they were going through (for their own use, donor, shared donor, embryo transfer), details about their retrieval and andrology information (sperm source and how the sample was collected). These sheets were kept for years following the patient's retrieval, because we also filled out embryo transfer details and pregnancy outcomes for each IVF patient, for every cycle they had. Many of the patients I saw at the clinic were pursuing IVF for one of three reasons: oocyte banking, embryo banking, or an ovulation disorder.

During the year that I worked at the clinic, we did not have any patients seeking treatment due to uterine factor infertility—even though it was listed under "Reason for Infertility" on our NASS worksheets. I think this may be because there are so few options for fertility treatments for women who are unable to conceive due to uterine factor infertility—especially in more severe cases. Undergoing an IVF cycle may help women

with other forms of infertility to conceive, but women with abnormal uterine pathologies may not benefit from only IVF in the same way. So, I chose to discuss the UTx procedure because I think there is a need for additional methods of treatment for women experiencing any form of uterine-factor infertility, as this appeared to be an underrepresented group at the clinic. As mentioned in Hur et al.'s review of uterine factor infertility, transplantation of functional uteri into patients diagnosed with total uterine dysfunction is currently the only protocol proposed to truly treat one of the most enigmatic forms of infertility in women (Hur et al., 2019). However, due to the long-term role that the uterus plays in fetal gestation, additional studies are needed to develop more reliable protocols for transplantation, postoperative care, and immunosuppressant usage.

Ovarian Tissue Cryopreservation

Within the field of reproductive medicine, reduced fertility as a result of ovulatory dysfunction accounts for roughly 40% of infertility cases worldwide (Castillón et al., 2018). In this section, I describe ovarian tissue cryopreservation, an experimental form of fertility preservation for female patients. I begin by describing forms of ovulatory dysfunction and reproductive cancer that would lead a patient to consider ovarian tissue cryopreservation. Next, I discuss how the procedure is performed, as well as some current studies documenting use of ovarian tissue cryopreservation. Finally, I describe how this method of fertility preservation would fit into the clinical setting where I worked, and future implications of the technique.

Ovulatory dysfunction is a multifaceted problem that can be caused by many different underlying conditions such as: PCOS, hyperprolactinemia, pituitary tumors, hypothyroidism, and numerous genetic disorders (Fluker & Fisher, 2007). Some mild

cases of irregular or absent menstrual cycles may be corrected through hormone therapy or surgical procedures. However, persistent ovulatory dysfunction often leads to patients relying on ARTs such as IVF in order to conceive, which begins with controlled ovarian stimulation.

Infertility related to ovulatory dysfunction can require alterations in traditional stimulation protocols and treatment plans used for IVF. For example, patients diagnosed with PCOS are at a much higher risk for developing ovarian hyperstimulation syndrome [OHSS] from stimulation medications used for IVF, which can be extremely dangerous for the patient (Stracquadanio et al., 2017). As a result, physicians typically use the antagonist stim protocol for PCOS patients to minimize the likelihood of hyperstimulation by using LH to trigger ovulation rather than hCG (Lin et al., 2014; Pundir et al., 2012). The antagonist protocol tends to require a shorter stimulation protocol and smaller doses of medications (Pundir et al., 2012). The LH trigger shot alone may not guarantee a surge sufficient to induce ovulation, which would result in no oocytes being retrieved (Beall et al., 2012). However, studies have shown that the LH-only trigger shot can reduce the likelihood of a patient experiencing hyperstimulation (Gunnala et al., 2017). As an intermediate option, some physicians may prescribe a combination of LH and hCG to trigger ovulation. Using a combination method reduces the risk of the patient developing OHSS but can increase the likelihood that the trigger shot will be sufficient to induce ovulation (Gunnala et al., 2017). These alternative methods to trigger ovulation should be evaluated on a patient-by-patient basis to ensure the best chance at successful ovulation without putting the patient at risk for developing OHSS.

Primary ovarian insufficiency [POI] is another severe disorder under the larger umbrella of ovarian dysfunction. Patients with a diminished number of oocytes remaining and who are entering menopause early have a limited timeframe in which they can undergo fertility treatment (Santoro & Cooper, 2016). Patients diagnosed with POI have often been described as "poor responders" during controlled ovarian stimulation for IUI and IVF, with fewer follicles responding to stimulation (Santoro & Cooper, 2016). For POI patients undergoing IVF, this means there are fewer oocytes retrieved during their cycle, which decreases the overall number of embryos generated for the patient. With fewer embryos, this limits the number of embryo transfers the patient can have, or the number of "chances" that patient has to conceive. Severe POI cases where no oocytes remain, or if the patient has experienced multiple failed IVF cycles will require the use of an oocyte donor in order to conceive.

In recent years, the experimental ovarian tissue cryopreservation [OTC] protocol has gained traction as a means of fertility preservation and restoration. OTC was initially developed as an emergency fertility preservation method for women diagnosed with cancer (Kristensen & Andersen, 2018). After receiving a cancer diagnosis, most reproductive-age women can undergo controlled ovarian stimulation to retrieve oocytes prior to beginning cancer treatment. This ensures that the patient still has a chance at having biological children post-treatment, even if their reproductive organs are compromised. Depending on the severity and location of the cancer, it may not be possible to delay treatment to pursue fertility preservation.

An urgent form of fertility preservation was needed to help patients preserve their fertility before undergoing life-saving cancer treatment, which led to the development of

the OTC protocol (Hoekman et al., 2020). One whole ovary or several small biopsies of ovarian tissue are taken from the patient, cut down into smaller fragments and cryopreserved using slow freezing. The protocol requires no stimulation of the ovaries and has negligible lead time compared to pursuing IVF treatment. In a study conducted by Shapira et al. in 2020, approximately 1,314 OTC procedures were performed between January 2004 (when the first OTC took place) and June 2018. Out of the 1,314 cryopreservation procedures conducted, only 70 patients had returned for re-transplantation. Within this small cohort, roughly 50.0% of OTC cases led to conception, and 41.6% resulted in live births (Shapira et al., 2020).

A second longitudinal case study conducted by Hoekman et al. followed 69 women between July 2002 to December 2015 who underwent OTC for fertility preservation (Hoekman et al., 2020). During that timeframe, 51 women participated in the follow up after 12 were lost to recurrence of cancer, and six were unable to be reached (Hoekman et al., 2020). Of the 51 remaining participants, seven women ended up having their ovarian tissue transplanted back, four of whom conceived and had at least one live birth after transplant (Hoekman et al., 2020). To date, over 130 live births have been reported following transplantation of previously cryopreserved ovarian tissue (Rivas Leonel et al, 2019 & Shapira et al., 2020). Despite many successful procedures, the "experimental" label of OTC has yet to be lifted. This is likely due to the small sample size of these studies. There is a wide margin of time between when a patient has ovarian tissue preserved and when the patient has the tissue transplanted again. Shapira et al. reported a median of 7 years between the time of OTC and transplantation, which does not include recovery time, nor the time to conception, pregnancy, and subsequent live

birth. Determining efficacy of the OTC protocol is highly variable because the history of patient follow up has spanned decades (Shapira et al., 2020).

The amount of time elapsed between the OTC procedure being performed and the tissue being re-transplanted is further exacerbated in OTC studies in children and young adolescents with cancer. Prepubertal children and adolescents are unable to pursue traditional methods of fertility preservation, as prepubertal ovaries do not respond to controlled ovarian stimulation (Arian et al., 2018). Based upon the initial success of OTC and re-transplantation in reproductive-age women, clinical studies are being conducted on the efficacy of tissue cryopreservation in prepubertal girls as well and is truly the only method for emergency fertility preservation in young girls (Christianson & Lindheim, 2018; Arian et al., 2018). Depending on the age of the patient at the time of their procedure, ovarian tissue may be frozen for several decades before being transplanted, meaning follow-up studies with these patients will take even longer. However, OTC has proven to be an emerging technique for fertility preservation for women in the studies conducted so far (Arian et al., 2018).

Past studies that have documented encouraging results of OTC in cancer patients have raised the possibility of other potential benefits of the procedure, especially for women experiencing other forms of ovarian dysfunction (Kristensen & Andersen, 2018). Other conditions that could benefit include benign ovarian tumors that would require removal of the ovaries, patients at risk of POI or who have genetic disorders related to POI, and patients with autoimmune diseases who need bone marrow transplants (Hoekman et al., 2020). Fragments of ovarian tissue can contain hundreds of primordial follicles (Bates & Bowling, 2012). While these primordial follicles are incapable of being

fertilized in their immature state, the process of follicular maturation has been replicated in vitro—meaning immature follicles can be induced to mature outside of the body to the point of being capable of fertilization, called *in vitro* maturation (Segers et al., 2020). *In vitro* maturation of oocytes followed by *in vitro* fertilization can generate viable embryos without the need for re-transplantation of ovarian tissue (Segers et al., 2020). The first live human birth using primordial oocytes matured and fertilized in vitro was recently reported in 2020 by Segers et al., demonstrating the true potential for ovarian tissue oocyte in vitro maturation. OTC combined with in vitro maturation and in vitro fertilization would not require a regimen of ovarian stimulation medications and might be ideal for patients with ovarian dysfunction that are poor responders or at higher risk for OHSS.

As I discussed in the previous section, one of the most cited reasons for infertility for patients undergoing treatment at my clinic was ovulatory dysfunction. For a fertility clinic, I view the potential benefit of OTC as two-fold. First, for patients with PCOS, fragments of ovarian tissue could be removed from one of their ovaries, and the lab could use an in vitro maturation protocol paired with IVF to yield embryos for the patient. The patient could then return to the clinic for their embryo transfer, avoiding the risk of developing dangerous symptoms of OHSS during traditional IVF. A second option is using OTC for patients with POI as a means of preemptive fertility preservation. These patients could undergo surgical removal of one of their ovaries, and we could cryopreserve their tissue until they were ready to conceive. This would effectively halt premature loss of their oocytes and give the patient more autonomy to choose when they would like to build a family. In the lab, we would hope to be able to extract primordial

follicles from the tissue when the patient is ready to begin trying to conceive, use in vitro maturation, IVF, and perform an embryo transfer.

At the clinic, we did have a protocol for in vitro maturation of oocytes; however, we did not provide that service in the lab. Immature oocytes were often discarded a day or two after the patient's retrieval if they did not reach maturity in vitro. If in vitro maturation becomes a more routinely offered service for patients, this might allow patients with immature oocytes to stand a better chance at having successful fertilization in the lab and improve cycle outcomes for patients with many immature oocytes. This could decrease the number of patients with no fertilized oocytes during their cycle—what we referred to as a "no fert." I believe the OTC protocol and use of in vitro maturation has implications beyond fertility preservation for cancer patients and has numerous other clinical applications. Use of these protocols at the clinic can potentially improve cycle outcomes for patients with other forms of reduced fertility.

Stimulating Spermatogenesis using FSH therapy

Analyses conducted on cases of infertility reported in the U.S. have shown that male-factor infertility is just as prevalent as female-factor infertility (Chandra et al., 2013). Although the burden of infertility is shared equally between both sexes, there is disproportionate emphasis placed on development of new therapeutic options for treating female factor infertility over male factor infertility. In this section, I discuss a novel form of fertility therapy that holds promise for stimulating spermatogenesis in men experiencing reduced fertility due to low sperm count. I begin by describing the various clinical categories of reduced fertility in male patients, focusing on reduced fertility caused by hormonal imbalance. Next, I briefly outline the role and importance of follicle-

stimulating hormone [FSH] in spermatogenesis, before discussing studies on FSH supplementation as a form of fertility treatment for male patients. I conclude by describing the ways I believe this form of fertility therapy will benefit patients in the clinic through a personal account of my time working as an andrologist.

In a clinical setting, men diagnosed with reduced fertility often experience one or a combination of conditions such as reduced sperm count (oligozoospermia), no sperm present in the ejaculate (azoospermia) or decreased sperm motility or quality. The results of a semen analysis are used to identify whether a patient can still use their own sperm to conceive using ART as a work-around for their reduced fertility, or if the patient will need to use donor sperm. Based upon the patient's medical history and test results, male infertility cases are grouped into one of three overarching categories: obstructive, sexual dysfunction, and non-obstructive infertility (Bhasin, 2007). Within each of these broad categories, numerous potential disorders and dysfunctions may be present, as described in Chapter 3. Most fertility clinics only provide treatments for women and the various forms of female-factor infertility. For a couple undergoing treatment at a fertility clinic, if the physician believes that the male patient's sperm count and quality is sufficient for IUI or an IVF cycle, he will not need to receive any treatment. However, if male-factor infertility is a substantial barrier to conception and the partner's sperm count and quality are extremely low, the male patient would be directed to a urology office for treatment before the couple could undergo IVF.

Urologists can more effectively diagnose reduced fertility in men, and in some cases, treat the underlying condition as well. The first category of reduced fertility that urologists diagnose and treat is obstructive infertility. Obstructive infertility encompasses

any form of infertility related to a blockage, including anatomical defects and blockage from scar tissue or previous infection (Raheem & Ralph, 2011). Impediment of the epididymis, vas deferens, or ejaculatory duct can cause low or zero sperm in the ejaculate and cause low semen volume as well—all factors that contribute to low fertility potential (Raheem & Ralph, 2011). Other conditions that may lead to obstructive infertility are unilateral and bilateral congenital absence of the vas deferens, which prevent sperm produced in the testes from mixing with the ejaculate (Bieth et al., 2020).

The second category of male infertility is infertility due to sexual dysfunction. Clinically, this form of infertility is referred to as "coital infertility." Examples of coital infertility include conditions such as premature and retrograde ejaculation, deformities of the penis, and erectile dysfunction (Bieth et al, 2020). Globally, sexual dysfunction is estimated to impact between 20-30% of all adult men (Lewis et al., 2010). However, only 2% of men have been diagnosed as infertile because of severe sexual dysfunction (Raheem & Ralph, 2011). Numerous health and psychological factors can lead to coital infertility, and they can be complex to effectively treat. The most common approaches to treating sexual dysfunction and coital infertility involve medications, psychological treatment, or a combination of both (Heiman, 2002; Lotti & Maggi, 2018).

The final category of male infertility is non-obstructive infertility. This category of infertility is broad and includes conditions such as varicocele, hormonal imbalance, genetics, abnormal development of internal and external reproductive organs, damage to the testes, and anti-sperm antibodies (Raheem & Ralph, 2011). Due to the limited number of treatment options available, non-obstructive infertility can be difficult to address. However, recent studies have shown promise for one particular etiology of non-

obstructive male infertility—hormonal imbalance. One of the first indications that male infertility may be caused by a hormonal imbalance is low sperm quality detected through a semen analysis. While there are sperm present in the ejaculate, there may be very few (oligozoospermia), and their shape, size, and movement are atypical. Reduced sperm quality is ultimately a reflection of defective or abnormal spermatogenesis. The process of sperm production is largely regulated by FSH, LH, and testosterone (outlined in Table 1, pg. 276). These hormones work both independently and in unison to promote production and maturation of spermatogonia during spermatogenesis (Betts et al., 2013). Levels of FSH, LH, and testosterone can also be used determine whether the patient has gonadotropin deficiency, primary testicular failure, spermatogenic failure or androgen resistance which can determine the method of treatment a patient can potentially receive (Bhasin, 2007).

FSH plays a particularly important role in the induction Sertoli cells in the testes which nourish and support developing sperm and also initiates the first meiotic division of primary spermatocytes (Betts et al., 2013). For FSH to actively induct Sertoli cells and initiate meiosis, FSH must bind to a specific receptor on the Sertoli cells called the follicle-stimulating hormone receptor [FSHR] (Oduwole et al., 2018). The FSHR is also present in women but can be found on granulosa cells, waiting binding of FSH to initiate development of ovarian follicles (Dierich et al., 1998). A single gene encodes FSHR, and it has been analyzed at the molecular level over the past several years to better understand the functional role of FSH and its receptor (Oduwole et al., 2018). Men with mutations in the gene that encodes FSHR or in the formation of FSH experience azoospermia or suppressed sperm production (Oduwole et al., 2018). The study conducted by Oduwole et

al. demonstrated the crucial role that FSH and its receptor play in development and maturation of sperm.

Given the current success with FSH to stimulate maturation of follicles in women for ART, researchers began to study the potential implications of FSH supplementation in men to stimulate spermatogenesis. FSH therapy was first tested as a method for restoring fertility potential in men diagnosed with hypogonadotropic hypogonadism [HH] (Santi et al, 2020). This condition can be congenital or acquired and is characterized by insufficient production of FSH and LH from the pituitary gland (Santi et al, 2020). Many patients with HH subsequently experience reduced non-obstructive infertility in the form of low sperm count or complete azoospermia (Santi et al, 2020). Supplementation through injectable FSH medications was expected to augment the body's natural cycling of hormones needed for spermatogenesis, ultimately increasing sperm production (Valenti et al., 2013). In a 2014 meta-analysis of 44 published gonadotropin therapy studies, gonadotropin supplementation in the form of FSH or as a combination of FSH/hCG demonstrated 69% overall efficacy in restoring spermatogenesis (Rastrelli et al., 2014). Use of FSH therapy has shown to be most effective in patients with low sperm count but less successful in men with complete azoospermia (Valenti et al., 2013). Most studies conducted using FSH supplementation were completed on a short-term basis of only 3 months. Studies reporting more significant results of gonadotropin therapy were conducted over the course of four months or longer—noting significant improvements in sperm concentration, motility, and morphology (Ding et al., 2015; Paradisi et al., 2006).

The favorable results of FSH therapy in male patients with HH have encouraged researchers to explore the possibility of using the same methods to treat idiopathic male

infertility. Due to the complex and enigmatic nature of idiopathic infertility, results of FSH therapy have not been consistent. A study conducted in 2017 concluded that after just one month of FSH therapy, sperm maturity had risen significantly in male patients with idiopathic infertility and documented marked improvement in sperm quality parameters for count, motility, and morphology (Casamonti et al., 2017). The study conducted by Casamonti et al. also reported even better results in these patients after three months of treatment. The following year, a correlational investigation conducted by Colacurci et al. noted an increase in sperm quality and reduction in DNA fragmentation of sperm following three months of FSH therapy (Colacurci et al., 2018). However, inconsistent with previous studies, a 2019 study revealed that only 40% of male patients regained normal semen parameters after three months of gonadotropin therapy and the remainder of patients studied saw no improvement in semen parameters at all (La Vignera et al., 2019). The current lack of consensus regarding validity of FSH supplementation merits further investigation. Inconsistent results can be due to numerous factors such as study design, sample size, or merely the unpredictable nature of idiopathic infertility. Further research is needed to determine if this treatment has potential for patients with idiopathic infertility.

Even for patients with HH, FSH therapy is not currently utilized as a routine method for treating reduced fertility. To compile a specific set of criteria to help identify male patients that may benefit from FSH therapy, more comprehensive analyses on both short- and long-term use of gonadal therapy are needed. Future studies may be improved through the implementation of pharmacogenomics—a field of medicine that studies how different individuals respond to medications based on their genetic makeup. Using a

patient's individual genetic information, researchers may be able to study variation of the FSHR-encoding gene and its individualized response to FSH therapy.

My training at the clinic began in the andrology department where I learned about the different parameters of a semen analysis and how abnormal values translated to various forms of male factor infertility. Thankfully for most patients, even if their sperm count was low or there were other abnormal parameters, we could still use very poor-quality samples and process them to be usable for IVF, and in some cases, for IUI as well. However, using IUI or IVF did not resolve the source of male-factor infertility, only bypassed it temporarily. Contingent upon additional long-term studies on the efficacy of FSH therapy as a form of treatment for low sperm count or quality, it would be beneficial for patients to have the option of having their natural fertility restored rather than undergoing multiple rounds of IUIs or IVF. If a couple was undergoing fertility treatment solely for male-factor infertility related to a hormonal imbalance, treating the root cause of infertility would be a much safer protocol than repeated rounds of controlled ovarian stimulation (and possible oocyte retrieval and embryo transfer as well for IVF) for the female patient.

Artificial Intelligence in IVF

The term "artificial intelligence" [AI] refers to a computer or machine's ability to emulate human thought. AI technologies are designed to execute complex functions using examples, experience, recognition, and decision-making. Using mathematical algorithms, actions and decisions made by AI are replicable and not influenced by human bias, except insofar as the programming reflects human input. But the developers work hard to overcome such bias. These features are particularly advantageous in the field of fertility

medicine—a field that still relies heavily on observations made by the human eye and are subject to cognitive bias. Bias in fertility medicine can be present within decisions regarding diagnoses, treatment plans, stim protocols, sperm quality assessments and embryo/oocyte grading.

In this section, I describe novel forms of AI in the early stages of clinical implementation in fertility medicine. The three major systems of AI that I discuss are the Embryo Ranking Intelligent Classification Algorithm, Life Whisperer AI, and Violet AI Software. These systems and programs are intended to reduce human bias in embryology, limit variation during testing, expedite diagnostic/treatment capabilities, and improve cycle outcomes overall by providing more reliable care to patients. I document the development of each system, discuss how these systems work and how they could have a positive impact in the fertility clinic laboratory setting, and describe my personal experience in the embryology lab and how we would have benefitted from systems such as these at our facility.

A study published in 2019 developed an AI system called the Embryo Ranking Intelligent Classification Algorithm [ERICA] to classify blastocysts using pattern recognition. The database used to train the ERICA system used still images of 1,231 blastocysts with a known outcome—either implanted successfully or failed to implant (Chavez-Badiola et al., 2020). These images were classified either “good quality” exemplary blastocysts that were euploid and did implant successfully, or “poor quality” blastocysts that were aneuploid or did not implant successfully (Chavez-Badiola et al., 2020). The model was trained to recognize what constitutes good quality versus poor prognosis based on the collection of example blastocyst images. To test the accuracy of

the ERICA system, senior embryologists were tasked with grading blastocysts based upon their knowledge and experience and comparing their results to the new system (Chavez-Badiola et al., 2020). ERICA was able to identify a "good prognosis" blastocyst in 78.9% of the cases—8.2% more often than the best-scoring embryologist (Chavez-Badiola et al, 2020). The system was also capable of grading four blastocysts in under 25 seconds, faster than the average embryologist, according to Chavez-Badiola et al. Based on this study, the ERICA system was able to rapidly grade embryos without compromising on accuracy, which is ideal for embryos, patients, and the embryologists.

For an embryologist, there is a delicate balance between speed and accuracy of grading. When working with human embryos, it is crucial to minimize the amount of time they spend outside of the incubator. Yet prioritizing speed over accuracy can lead to inaccurate embryo grading. Embryos are 3-dimensional structures that are depicted as only a 2-dimensional image when viewed under a microscope. Embryos are graded based on the number and uniformity of cells present, the presence of their cytoplasm, degree of compaction, presence of vacuoles and fragmentation, amount of expansion or hatching, and quality of the inner cellular mass and trophectoderm (Lewis, 2020). Assessing each of these variables as quickly and accurately as possible takes a lot of practice. A newer embryologist may be slower to assign grades or may give harsher gradings because they don't want to give a generous grading and have the embryo they deemed "good quality" not survive or not implant successfully in a patient. Variations in training from clinic to clinic can also lead to embryos being graded differently. This can be problematic because patients work with embryologists to determine which embryo they should transfer and will often starting with the embryo of best quality. If an embryo is given an inaccurate

grading because the process was rushed to minimize time out of the incubator, a patient may transfer or discard embryos with grades that do not reflect their actual quality. Ideally, AI systems will be improved to use time-lapse imaging to assess embryos in real-time rather than static 2-dimensional images. However, even the current capabilities of automated embryo-assessment have great potential, despite its limitations.

Similar to the automated grading system implemented through ERICA, improved embryo selection is also being optimized by Life Whisperer, a newly commercialized technology that utilizes AI to assess viability of embryos (Perugini, 2020). The Life Whisperer application is designed to serve as a "clinical decision support tool that removes subjectivity and provides an additional objective assessment of embryo quality—to make the best decision about the order of embryos to transfer" (VerMilyea et al., 2020). The AI system was trained using still, 2-dimensional images of nearly 400 day-5 embryos with known pregnancy outcomes (VerMilyea et al., 2020). According to VerMilyea et al., the Life Whisperer model showed 70.1% accuracy in predicting embryos with positive pregnancy outcomes and 60.5% accuracy for embryos with negative pregnancy outcomes.

Through establishing morphological patterns seen in normal embryos, Life Whisperer technology has been able to detect morphological features associated with abnormalities in chromosomes 16 and 21 in day 5 embryos (VerMilyea et al., 2019). Genetic diagnostics using embryo biopsy is currently the only method for screening embryos for chromosomal abnormalities, which is costly and invasive to the embryo. Using AI to non-invasively detect genetic anomalies in embryos has the potential to be far more cost-effective, safe, and efficient than traditional genetic screening. Additional

studies being conducted using Life Whisperer AI are intended to increase precision of the system and investigate other chromosomal abnormalities that may be associated with embryonic morphology (VerMilyea et al., 2019).

As a result of initial success using the ERICA and Life Whisperer systems for grading embryos, there is now speculation that AI can eventually be utilized to address a largely unmet need in embryology, specifically standardized oocyte assessment. Unlike embryo grading, there is no current scoring system for assessing oocyte quality (Wang & Sun, 2007). Oocytes can be generally regarded as normal or abnormal based on their shape, size, and cytoplasmic/extra-cytoplasmic characteristics (Wang & Sun, 2007). However, the lack of any widely accepted scoring system means that many clinics do not spend time performing oocyte quality assessments. From a clinical standpoint, mature oocytes are treated as if they all possess the same fertilization potential. But in reality, oocyte quality is multifaceted and highly individualized, and not all oocytes have an identical likelihood to fertilize successfully. There is no universally accepted standard in embryology for assessing oocyte quality based upon morphological features (Wang & Sun, 2007). Despite the complexity of quality determination, knowing the quality of each individual oocyte can be indicative of fertilization potential (Krisher, 2004). Therefore, developing and implementing a standardized method for assessing oocyte quality would be a significant advancement in fertility medicine. This not only applies to patients using their own oocytes, but also for patients using egg donors as well. Once a reliable method of oocyte assessment is established, patients seeking donor oocytes can review quality data and select oocytes with the highest fertilization potential.

Finally, Future Fertility is a Canadian startup company that has recently begun piloting their AI software named Violet, which functions similar to the ERICA system. Violet is designed to rapidly and non-invasively assess oocytes for quality and provide instantaneous feedback to embryologists, clinicians and even patients (Nayot, 2020). The Violet system uses 2D images to provide nearly instantaneous feedback regarding oocyte classification, and prediction of embryo implantation success (Nayot, 2020). Violet has been tested in over 20,000 cases with known outcomes and demonstrated 91.2% accuracy in predicting fertilization, and 99% accuracy in predicting negative or unsuccessful fertilization (Nayot, 2020). Nayot also reported that in comparison to assessments made by unaffiliated senior embryologists, the Violet system was over 21% more accurate in predicting both fertilization and blastocyst outcomes and completed 300 oocyte assessments in under five minutes versus 1-2 hours required by embryologists.

Employing AI systems such as ERICA, Life Whisperer, and Violet in the clinic can come with some up-front costs for installing specific programs. However, these AI systems have been developed to be compatible with the light microscopes and desktop computers commonly utilized in fertility clinics today. Clinics will not be required to additionally invest in new equipment to bring AI into their labs. Automated methods for assessing grades and viability of oocytes and embryos will not only create a consistent standard unaffected by individual bias, but it will also save time, minimize cellular stress, and provide both rapid and invaluable information regarding potential cycle outcomes before fertilization and embryo transfer have taken place.

My clinic performed between 60-80 IVF retrievals each month which were typically conducted within a two-week span. This meant there were hundreds of oocytes

and embryos present in our lab during some of our busiest points in the month. Given the numerous responsibilities of the embryology staff that include quality control assessments, embryo, and oocyte dish preparation, performing retrievals, fertilizing oocytes, assigning embryo grades, culturing, recordkeeping, reporting, biopsying, freezing, and transferring, the embryologist could greatly benefit by having any part of this process automated and with high accuracy. Understaffed laboratories that function with only two or three embryologists and experience waves of high patient volumes would also benefit. Additionally, busy fertility clinic laboratories such as the lab I worked with could begin giving both oocytes and embryos quality grades without compromising on accuracy or requiring more time.

With technologies such as these, oocyte and embryo grades could be directly passed on to the physician and patients, relieving the embryologists from having to scan in embryo grading sheets for patients or calling patients to inform them of the status of their embryos. Everything could be done seamlessly and allow for a more continual feed of information about a patient's embryos. Many times, during a busy cycle, a patient would call the clinic and ask to speak with someone from the lab about "how things are looking" with their embryos. With a limited number of staff, we relied heavily on medical assistants and nurses to relay information about embryos to patients if we had not had the chance to upload a report to the patient's profile yet or couldn't take the patient's call at that moment. I believe that implementation of AI in fertility can improve transparency between laboratory staff and patients by keeping the patients more informed and involved throughout the IVF process.

At-Home Fertility Testing

Options for testing and monitoring fertility outside of the clinic have been around for many years, largely aimed towards giving women greater autonomy with their own reproductive health. In this section, I cover methods of at-home fertility testing available for both men and women. I focus on the three overarching goals of at-home testing—accuracy, affordability, and convenience, and analyze if and how different companies accomplish those goals. I address remote fertility testing for women first, discussing hormone testing services provided by Modern Fertility, EverlyWell, and LetsGetChecked. Then, I describe the different forms of fertility testing available for men, grouped by the service they provide. These include remote fertility hormone testing, semen analysis, or sperm cryopreservation. For each company and service, I describe how their testing works, how they report results and communicate those results to patients, and the cost associated with each service. I conclude by providing my thoughts about the benefits of offering and accepting remote fertility testing based on the interactions I had with patients during my time at the clinic.

The first methods of at-home fertility monitoring were techniques associated with natural family planning which include monitoring basal body temperature, noting changes in cervical mucus, and using urine-based test strips to detect presence of LH and hCG (Manders et al., 2015; Miller & Soules, 1996; Gnoth & Johnson, 2014). The primary benefit of at-home testing has been convenience. Test strips can be found at grocery stores, pharmacies, convenience stores, and even ordered online and delivered straight to the patient's home. Yet the convenience of these tests comes with the cost of low accuracy. The accuracy of results from qualitative test strips and self-monitoring are

not as reliable as quantitative tests that use blood samples to detect various concentrations. While qualitative tests may work for patients with no source of reduced fertility, the information provided by self-monitoring and other qualitative testing methods may be insufficient to help a couple conceive.

At the clinic, ovulation and pregnancy are monitored through quantitative blood-testing and ultrasonography. These more accurate methods of diagnostic fertility testing require a trip to a fertility clinic or outside lab and are considerably more expensive than their drugstore alternatives. There are undeniable pros and cons of at-home fertility tests versus in-house diagnostic fertility testing. Over time, the availability of options has created a demand for new methods that meet all the desired criteria of fertility testing: accurate, affordable, and convenient testing for both women and men. With the many technological advancements used in fertility medicine today, developing a novel form of testing that meets these criteria sounds like it would be relatively easy to carry out. However, numerous challenges arise with accomplishing all three goals.

This novel fertility testing service must provide detailed, accurate reports that can be easily understood by the patient. Results must be securely stored in digital form, and able to be transmitted to a patient's healthcare provider if they decide to pursue treatment at a clinic. Directions for collecting a sample for the test need to be simple yet effective. The patient needs to be able to independently collect the sample without a face-to-face consultation. After the sample is collected, it must be stored in packaging that keeps the sample stable as it is shipped through various environmental conditions before being received and analyzed. Finally, the results of the testing need to be reliable and presented

in a format that can be understood by fertility clinics nationwide if the patient decides to seek treatment at a clinic.

Tasked with developing a superior method to assess fertility, companies such as Modern Fertility, EverlyWell, and LetsGetChecked have developed a series of at-home test kits that assess a wide range of reproductive health aspects, namely fertility potential and ovarian reserve testing. Since blood samples are the preferred method for analyzing fertility hormones, the first hurdle for developing an at-home test kit was to develop a method for patients to safely collect a blood sample and preserve it for shipping. Single-use lancets, or finger-prick devices similar to those used for monitoring blood glucose are provided for the patient to create a small cut in the skin. This allows a small blood sample to be collected and is far less invasive than traditional venous blood sample collection. Drops of the patient's blood are placed on a marked collection card. Once the sample collection card is full, the blood is allowed to fully dry before being sealed in a biohazard bag for shipping. The sample is shipped to a certified lab for analysis and the patient's results are uploaded into a secure portal.

The cost of at-home fertility testing ranges from $130-$150 depending on the company and number of hormones being analyzed. By comparison, having a full panel of fertility testing conducted by a clinic may cost upwards of $1,000, so there is a substantial difference in the cost of testing. Modern Fertility provides the largest number of potential hormones that can be analyzed: AMH, FSH, Estradiol, Prolactin, TSH, Free/Total T4, and LH. Prior to ordering the kit, Modern Fertility asks a series of questions to determine which hormones should be analyzed, which can vary based upon age, medications, and the form of birth control being used by the patient. Regardless of

the number of hormones being measured, the overall price of the test remains the same and each patient receives results through the Modern Fertility dashboard. Results can be tracked over time, which is reported to "help people understand how their fertility changes over time so that they can take action and be proactive about their fertility planning" (Modern Fertility, 2021). Patients are given access to an online forum of other Modern Fertility users and provides one-on-one consultations with a fertility nurse. Both of these features are unique to Modern Fertility.

EverlyWell's fertility test functions in a similar way but is slightly less expensive and measures fewer hormones: Estradiol, LH, FSH, TSH and Total Testosterone. There is no customization of the EverlyWell fertility test—all five hormones are measured and reported. Patients are given access to their results online but receive no consultation regarding the meaning of their results. Instead, the patient can share their EverlyWell results with their physician to analyze what their individual results mean. For a greater selection on testing kit packages, LetsGetChecked offers three different tests: Progesterone, Ovarian Reserve, and Female Hormones. The progesterone test only measures levels of progesterone which may be adequate for determining whether ovulation has occurred. The ovarian reserve test kit solely measures AMH levels to assess the patient's ovarian reserve. The final kit is the female hormone kit, which analyzes FSH, LH, prolactin, and estradiol. For comprehensive testing, an initial workup may require multiple kits from LetsGetChecked which is not as cost-effective as the Modern Fertility or EverlyWell kits. Various packages provided by LetsGetChecked may be sufficient for patients repeating results or who are on birth control and not actively trying to conceive.

Currently, these at-home fertility tests for women are advertised as "proactive wellness products" designed to inform patients of their reproductive health but not to replace the comprehensive diagnostic capabilities of a fertility clinic. Patients are still encouraged to consult a physician and discuss their results and treatment options if necessary. Laboratories that analyze samples from Modern Fertility, EverlyWell, and LetsGetChecked are all accredited by both the College of American Pathologists [CAP] and Clinical Laboratory Improvement Amendments [CLIA], meaning all are held to the same federal standards that fertility clinics are held to in the U.S. Results garnered through at-home fertility testing for women are becoming more commonplace and may provide sufficient information for couples to conceive naturally, as long as no source of reduced fertility is detected. Patients with suboptimal results from at-home fertility testing that subsequently struggle to conceive may still be asked to have in-house diagnostic fertility testing completed at the clinic, as not all physicians accept at-home fertility testing as an acceptable baseline. As more patients use these methods for independently assessing their own fertility, more physicians may begin to accept reports from at-home fertility tests as a part of a patient's comprehensive medical history which can ultimately save patients money up front in the early diagnostic phase of their infertility treatment.

Most at-home tests and applications used to monitor fertility are marketed towards women, leaving male-factor infertility largely unaddressed outside of a scheduled visit to a clinic. However, some men feel uncomfortable visiting a fertility clinic and may struggle to collect an adequate sample for analysis at the clinic (Parekh et al., 2020). The present need for accessible methods of self-monitoring male fertility has

begun to be addressed in similar ways, prioritizing convenient, secure services to assess male hormones, sperm quality and even store sperm for future use. LetsGetChecked (blood-based) and EverlyWell (saliva-based) have testosterone-only test kits available which cost between $50-$70. In most cases, testosterone is the only hormone measured in relation to male fertility.

The most essential test for male fertility is the quality and quantity of sperm present in their ejaculate. At a clinic, patients can expect to spend $125-$268 for a complex semen analysis. SpermCheck Fertility is the cheapest method for at-home male fertility testing which retails for $40 and is similar to urine-based pregnancy/ovulation tests (Parekh et al., 2020). The SpermCheck fertility test is a single-use device that tests a small sample of semen to detect a sperm count of >20 million/mL. Drops of semen are loaded into a single-window device that is read the same way as a urine-based pregnancy test: a visible control line and test line indicate a positive result of >20 million/mL (DNA Diagnostics Center, 2020). Comparable to the way the SpermCheck fertility testing works, the Trak Fertility device comes as a packaged set that also detects sperm count using a small plastic device, retailing for $90, or $45/test (TrakFertility, 2019). Trak Fertility uses a small centrifuge-like device to condense sperm into one end of the device and has readable lines that indicate the estimated sperm count (Parekh et al., 2020). Sperm count is a valuable piece of information that can indicate male fertility potential but is just one of many parameters analyzed in a standard semen analysis. SpermCheck and Trak provide no information regarding quality of sperm in the semen sample, which has very limited use for fertility applications.

For a more detailed view of a patient's sperm, YO home sperm test uses a mobile application and desktop connection to analyze a sample of semen (YO Home Sperm Test, 2021). The test kit is only available online and includes the YO device and supplies for two semen tests for $80 (YO Home Sperm Test, 2021). The patient collects a sample at home and loads a small volume into the provided slide. After connecting YO to a computer, the slide is inserted into the YO device. The YO device functions like a small microscope to view the sample and applies algorithms to calculate motility and concentration of the sample, provided as numerical values (Agarwal et al., 2018). A 2018 study conducted by the Cleveland Clinic revealed that YO demonstrated a high level of accuracy and correctly identified normal/abnormal sperm motility, and below-threshold sperm concentration (Agarwal et al., 2018). YO also saves a video of the patient's motile sperm which can be referenced by a physician during consultation for treatment (YO Home Sperm Test, 2021). Although the test does have limitations. While the YO sperm test does provide significantly more detail than simple qualitative concentration tests such as SpermCheck and Trak, there are no details on sperm morphology which is an important factor taken into consideration when suggesting a patient pursue IUI or IVF treatment at the clinic. Without evaluating morphology, physicians cannot definitively assess whether ICSI must be used for fertilization in IVF cases. However, YO Home Sperm Test could be a valuable first step in a patient's decision to seek fertility treatment in the first place.

The most comprehensive at-home fertility tests for men are produced by Fellow, Legacy, and Dadi. For each company, the patient orders a complete kit online that is delivered to their home. Standard procedure for these kits include the patient collecting a

sample into the labeled specimen cup, mixing a provided preservation solution with the sample, sealing the cup and shipping it off to the company's CAP/CLIA accredited lab for analysis. Once the sample has been received by the lab, the patients should receive an electronic copy of their semen analysis results within 24-72 hours (Fellow, 2021). Fellow offers an at-home complex semen analysis for $189, which includes a detailed report documenting the sample volume, concentration, total/motile sperm count, motility, and morphology (Fellow, 2021). Results are authorized by a licensed physician and patients have the option of scheduling a virtual consultation with Fellow if they need assistance understanding their results.

Dadi and Legacy provide identical services for mail-in complex semen analyses, but also offer sperm cryopreservation services. Patients collect and mail in their sample using a kit, the sample is analyzed in an accredited lab, and depending on the quality of the sample, Dadi will freeze up to 3 vials of sperm, and Legacy will freeze up to 4 vials for the patient (Dadi, 2021; Legacy, 2021). A basic collection kit from Dadi costs $199 and includes a basic fertility report, 3 vials of sperm stored free for 1 year (Dadi, 2021). For every year after, Dadi charges $99/year for storage, with a $300 sample withdrawal fee (Dadi, 2021). Dadi does not offer fertility testing without storage, so their services are not recommended for patients looking for a fertility report alone. Features of the premium packages by Dadi include advanced fertility reports with nursing consultations, and additional vials stored (Dadi, 2021). Legacy's pricing is based off urgency and need, for example—how long will the patient need their sample stored? For $195, Legacy will conduct a complex semen analysis and store the patient's sample for one week or offer a monthly or annual plan at $14.95/month or $145/year (Legacy, 2021). For additional

deposits and long-term storage, prices range from $995-$3,995 depending on the patient's timeline (Legacy, 2021). Legacy does not offer fertility testing without storage, however, the month-to-month payment option for cryogenic storage with Legacy makes their services more affordable for patients that only need storage on a short-term basis.

Options for at-home fertility tests have grown substantially over the past several years, for male and female patients alike. I believe that as remote fertility testing becomes more widely available and accepted at fertility clinics, the benefits will be two-fold. The first benefit is that patients will be able to take a first step towards understanding their reproductive health from the comfort of their home. For patients who want to understand their fertility but not visit a clinic and set up multiple appointments, remote fertility testing is a great option. During my time at the clinic, I noticed that some patients felt ashamed about their infertility and did not feel comfortable talking about it, even with healthcare professionals. Using remote fertility testing, patients can begin to take steps towards beginning treatment at their own pace.

The second benefit is for the clinic staff. Better integration between applications, remote fertility testing services, and patient healthcare profiles can allow patients to share fertility testing results with their clinic of choice before their consultation with the physician. This can streamline the diagnostic process since the patient has already had various fertility tests performed. Even if the patient needs to have the values repeated at the clinic for verification, previous test results using at-home fertility testing can confirm a diagnosis more quickly. With more reliable and comprehensive at-home fertility testing, patients can be more proactive in their reproductive health, and physicians can more quickly diagnose and treat new patients at the clinic.

Conclusion

Continual efforts made by researchers have contributed to the development of new therapies, treatments, and technologies in the field of reproductive medicine. Each of the research topics and new technologies I discussed in this chapter contributes to the two overarching goals of reproductive medicine: to treat patients experiencing reproductive dysfunction and to improve our ability to diagnose forms of reduced fertility more efficiently and effectively. I described the individual contributions of each to the field of reproductive medicine at large, as well as the clinic where I worked. I believe offering new services and improving upon the services currently offered at fertility clinics will enhance our ability to provide high-quality, comprehensive care to patients.

CHAPTER 7

ROLE OF TYRAMINE IN THE MOUSE OVARY

Synopsis

Until this point, the content of my dissertation has focused on reproduction in humans. I drew upon extensive literature review and personal experience working at a fertility clinic to describe how reproduction works, how we diagnose and treat reproductive dysfunction in a clinical setting, and what research is being implemented in humans to advance the field of reproductive medicine. While implementation in humans is the long-term goal, the path from an idea to an approved therapeutic begins long before humans are a part of the equation. Biomedical research begins with *in vitro* or *in vivo* pre-clinical trials, most often using animals as research models. Animal studies play an integral role in the development of new therapies and treatments, as well as demonstrating efficacy and safety.

My initial interest in reproductive medicine developed through animal research I conducted at Arizona State University. In this chapter, I describe the project that jumpstarted my interest in reproductive medicine—my animal study on the biogenic amine tyramine and its role in mouse ovulation. I describe the methods I used to compare the effects of tyramine on follicular maturation and ovulation, specifically High-Performance Liquid Chromatography, histology, confocal microscopy, and immunohistochemistry. Finally, I cover the key findings during my experiments and the future directions and implications of my study.

Introduction

Complications with ovulation are associated with nearly 40% of infertility cases in women each year, yet little is known about the causes of ovarian dysfunction (Castillón et al., 2018). Ovulatory disorders typically manifest as irregular or absent menstrual cycles that make it difficult for a patient to become pregnant. Problems with ovulation are more commonly seen in conjunction with polycystic ovarian syndrome [PCOS], primary ovarian insufficiency [POI], hyperprolactinemia, malfunction of the hypothalamus, and other risk factors that can negatively impact an individual's ability to conceive. (Palomba et al., 2009; Santoro & Cooper, 2016). Patients who struggle to conceive naturally for an extended period may choose to pursue treatment at a fertility clinic. At the fertility clinic, treatment often begins with medicated ovulation induction prior to undergoing intrauterine insemination, or an *in vitro* fertilization cycle.

Through oral and injectable medications, medicated ovulation induction can be used to stimulate one or more follicles in the ovaries to mature simultaneously. However, ovulation induction protocols are not ideal for all patients, and will not be effective for all forms of infertility. Since ovulatory dysfunction is an umbrella term that manifests in numerous ways, there's no "one size fits all" stimulation protocol that works for all forms of ovulatory dysfunction. For example, patients diagnosed with PCOS are at a much higher risk for developing ovarian hyperstimulation syndrome [OHSS], a condition that can be extremely dangerous or even fatal for the patient if left unaddressed (Stracquadanio et al., 2017). Similarly, patients experiencing POI may be poor responders to stimulation medications and have very few follicles respond to stimulation, if any at all (Santoro & Cooper, 2016). Complications associated with ovulation can be

multifaceted, therefore, developing a more comprehensive understanding of the signaling pathways involved in the regulation of ovulation may reveal new methods for controlling ovulation—specifically for women with anovulatory or irregular menstrual cycles.

As previously described in Chapter 2: Reproduction: An Overview, female reproductive physiology is regulated by through feedback loops involving numerous hormones and molecules that tightly control maturation and rupture of follicles in the ovary, maintenance and shedding of the uterine lining, and implantation of an embryo during conception. The first feedback loop that influences reproductive processes is the hypothalamic-gonadal-pituitary axis. The hypothalamus is responsible for the production of gonadotropin-releasing hormone [GnRH] (Hill, 2019). GnRH then acts on the pituitary gland to begin the release of luteinizing hormone [LH] and follicle-stimulating hormone [FSH] (Hill, 2019). Other hormones such as estrogen and progesterone are predominantly produced by the uterus and ovary, respectively (Betts et al., 2013). The second feedback loop is the hypothalamic-pituitary-adrenal axis, which is responsible for production of prohormones, peptides, and synthesis of catecholamines (Nussey & Whitehead, 2001). Crosstalk between these two axes can up-regulate or down-regulate production of hormones and molecules, which is essential for normal reproductive function.

Catecholamine biosynthesis is an integral function of the hypothalamic-pituitary-adrenal axis (figure 15). During catecholamine biosynthesis, dopamine, tyramine, octopamine, and norepinephrine are all derived from a precursor of tyrosine (Nussey & Whitehead, 2001; Cole et al., 2005). One of the many molecules derived from the amino acid tyrosine is Tyramine. Tyramine is a small, volatile molecule derived from tyrosine as part of the catecholamine biosynthesis pathway. Throughout history, the majority of

studies that have examined the effect of tyramine have been conducted in invertebrate insect species (Evans, 1980; Roeder 2005; Roeder et al., 2003). Studies involving vertebrate species, specifically humans, were more limited due to the heightened complexity of hormone regulation. A foundational study in humans conducted in 1982 reported that tyramine induced peripheral vasoconstriction, increased cardiac output, and increased respiration in patients with hypertension—factors that are each connected to the activity of smooth muscle (Bianchetti et al., 1982). The results of the study conducted by Bianchetti et al. simultaneously raised additional questions about future use and implications of tyramine in other areas of the human body.

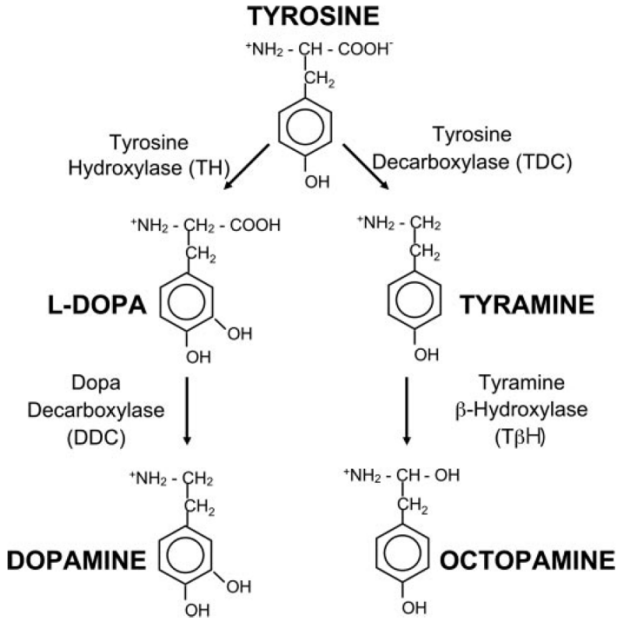

Figure 15. Catecholamine Biosynthesis Pathway. This figure illustrates the synthesis of tyramine from its precursor tyrosine during catecholamine biosynthesis (Cole et al., 2005).

Within the last ten years a few additional studies have revisited the role that tyramine may play within the human body. Most of these focused on the role of tyramine in the diet. Elevated levels of tyramine can be introduced to the body through diet by consuming large amounts of aged meat, soy proteins and cheese. The body naturally breaks down proteins through metabolism into amino acids. The amino acid tyrosine is in large quantities especially in cheese. Tyrosine is further broken down into biogenic monoamines such as tyramine which was discovered to cause hypertension. This condition became well known as the "cheese effect." The body will normally break down tyramine using an enzyme called monoamine oxidase [MAO] but this can be inhibited in individuals who are treated with a monoamine oxidase inhibitor [MAOI] that is prescribed for anxiety, depression, Parkinson's disease, and bipolar disorder (Finberg and Gillman, 2011). In recent years, because of the advancement of new research tools and advancements in genomics, researchers began to examine the role of tyramine throughout the body. One study focused on the male reproductive system in rats and found that elevated levels of tyramine could induce continuous peristalsis of the vas deferens (Finberg and Tenne, 1982). More recently, a study conducted by Obayomi et al. in 2017 demonstrated that the biogenic monoamine tyramine was capable of inducing contractions of uterine smooth muscle in mice at levels comparable to estrogen (Obayomi et al., 2017). These studies not only revisited the role of tyramine, but also proposed that tyramine could act as a modulator of reproductive function in mammals such as rats and mice. This research inspired me to study the role of tyramine in mouse ovulation.

Ovarian follicles possess numerous cell types that share common morphological features with smooth muscle such as the tunica albuginea, chordae, and theca externa,

illustrated in figure 16 (Peck et al., 2019). These smooth muscle-like cells play a role in the cycling of oocytes within the ovary, and ultimately their expulsion from the follicle. Smooth muscle contraction is modulated by many sources such as neurotransmitters (i.e. dopamine), hormones (i.e. epinephrine) and chemicals (i.e. nitrous oxide) (Obayomi et al., 2017).

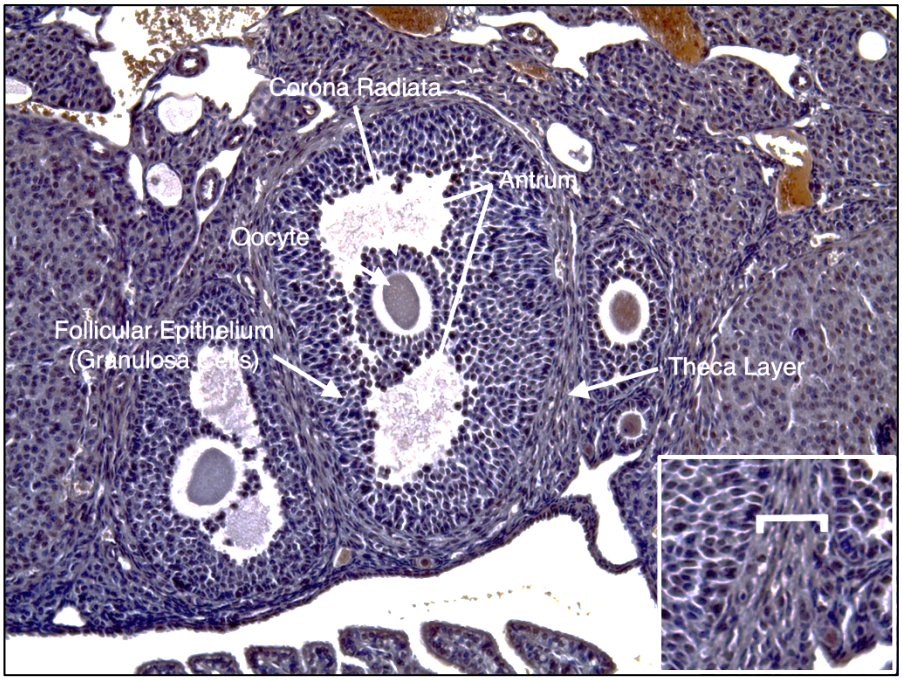

Figure 16. Structural Elements of the Ovarian Follicle. Histological Section of Secondary ovarian follicle stimulated with PMSG, with major structural elements labeled. This section is labeled with TAAR1 (brown) and counterstained with Hematoxylin (blue). Insert: Detail of theca layer versus follicular epithelium. Imaged using EVOS Imaging System using 20x Objective.

Since tyramine can modulate uterine contractions, my project investigated what affect tyramine may have on the ovary and ovulatory process, based on the shared morphological similarities between ovarian follicles and smooth muscle. To explore this hypothesis, I conducted research using mice (*Mus Musculus)* as an animal model to garner further insight into the regulatory mechanisms of ovulation.

Results

My study analyzed the role of tyramine in mouse ovaries using High-Performance Liquid Chromatography, histology, confocal microscopy, and immunohistochemistry protocols described in section 7.5: Materials & Methods. I analyzed ovaries from five different experimental conditions: non-injected, non-injected + tyramine, PMSG (pregnant mare serum gonadotrophin) only, PMSG + tyramine, and complete super ovulation using PMSG and hCG (human chorionic gonadotropin). Non-injected ovary samples served as my negative control, to demonstrate the normal state of the ovary with many follicles at various stages of development and maturation. The remaining samples received *in vivo* injections, *in vitro* treatments, or a combination of both injections and treatments. Ovaries isolated from mice that received PMSG only and those who had undergone complete superovulation were used to make comparisons with my samples that had been treated with tyramine. The importance of the PMSG only and completely super-ovulated samples was to show the changes in ovarian morphology after the first and second injections used in standard superovulation protocols—demonstrating what is "normal" during the process of superovulation.

In the fourth experimental condition I examined non-injected ovaries that were treated with tyramine *in vitro*. Observing the effect of tyramine on its own without any

hormonal injections was important to establish an understanding of whether tyramine would have an effect on its own, and if there was an effect, the extent tyramine influenced follicle rearrangement in the ovary. My final experimental condition was PMSG + tyramine. For these samples, mice were injected with PMSG only, and euthanized 48 hours afterwards. The ovaries taken from these mice were treated with tyramine *in vitro*. Examining ovarian samples that had been partially super-ovulated before the addition of tyramine allowed me to determine if tyramine influenced follicle maturation during the middle of standard superovulation protocol, or if tyramine had a stronger effect on its own.

Superovulation using injections of PMSG and hCG initiate rapid maturation of oocytes from meiotic metaphase I to meiotic metaphase II (Peck et al., 2019). After the follicle reaches maturity, the follicle border ruptures, expelling the oocyte into the oviduct. During superovulation, a mouse ovary can release between 6-16 oocytes at once (Silver, 2000). With numerous follicles rupturing and collapsing during ovulation, this left the ovary with a deflated appearance, as demonstrated in Figure 17C. Using confocal microscopy, I was able to identify regions specifically labeled in the mouse ovary for tyramine's receptor, Trace Amine-Associated Receptor 1 [TAAR1]. In non-injected mouse ovaries, the distribution of TAAR1 appears uniform throughout the sample (Figure 17A). However, upon stimulation with PMSG, hCG, and/or tyramine, TAAR1 localized in the corpus luteum of ruptured follicles and the perimeter of the ovary where oocytes had been released during ovulation (Figure 17B-D).

Figure 18 shows a cross-section of a non-injected ovary (A) and a non-injected ovary treated with tyramine (B) that has been labeled with a phalloidin dye that

selectively binds to actin, the primary component of smooth muscle. Based upon the initial results of my immunohistochemical labeling of TAAR1 in ovarian sections, I predicted that stimulating ovarian tissue using a physiological dose of tyramine could alter the organization of follicles within the ovary and potentially initiate ovulation (Peck et al., 2019). Following exposure to tyramine, follicle borders did appear disrupted (Figure 18B) in comparison to the control (Figure 18A), and there were more tertiary/final-stage follicles present in tyramine-treated ovaries than observed in the experimental control. Using a similar technique that produces thinner histological sections, I am able to stain the entire tissues to more clearly visualize the ovarian tertiary follicles (Figure 19). In both completely super-ovulated mice and mice exposed to PMSG and tyramine, localization of TAAR1 was enriched within each individual follicle and in the oocyte itself (Peck et al., 2019). Additionally, the TAAR1 receptors were found to localize predominantly near tertiary follicles. The collective findings between my immunohistochemical and histological data suggests that tyramine might play an active role in the process of follicular maturation and rupture in mice.

Finally, I quantified the levels of tyramine in the ovary using High Performance Liquid Chromatography [HPLC]. This method uses a column that can separate, identify, and quantify select molecules in a sample. Non-injected and completely super-ovulated (injected) ovaries were prepared into a solution and injected through a column to quantify how the levels of tyramine present in the tissue changed before and after superovulation. HPLC analysis demonstrated that non-injected ovaries contained higher levels of tyramine than ovaries that had undergone superovulation using PMSG/hCG (Figure 20)

(Peck et al., 2019). The results obtained using HPLC provided valuable insight into the potential way that tyramine may be utilized during the ovulatory process in mice.

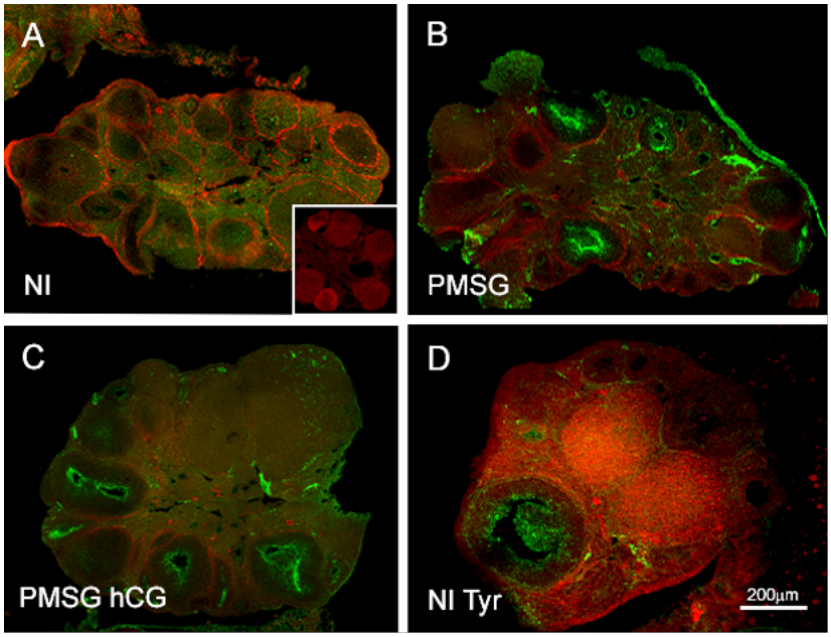

Figure 17. Localization of TAAR1 in Mouse Ovaries. Mouse ovaries are either non-injected (A), injected with PMSG only (B), completely super-ovulated using both PMSG and hCG (C), or post-treated with a physiological dose of tyramine after ovary removal (D). Actin within follicle borders is labeled with phalloidin (red). TAAR1 localization is visualized in green, showing localization around the perimeter of each follicle, and enriched in the corpus luteum.

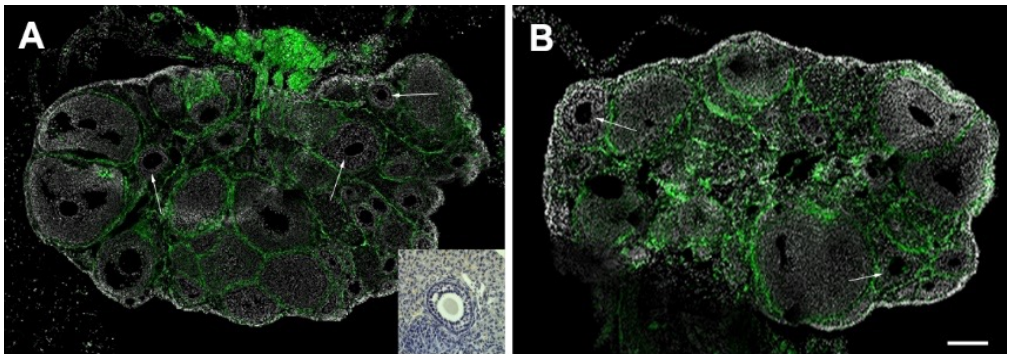

Figure 18. Effect of Tyramine on Mouse Ovaries. Image A shows a cross section of a non-injected ovary. Follicles in image A are at various stages of the maturation process, with actin/phalloidin labeling in green and DNA/DAPI in gray. Image B shows a cross section of a non-injected ovary that was exposed to a physiological dose of tyramine for 45 minutes *in vitro*. The ovary in image B that was stimulated with tyramine shows a disruption of follicles that resembles the appearance of an ovary that has undergone complete superovulation. The inset in image A is a histological preparation showing the usual pattern of DNA organization around a primary follicle. Scale bar is 200μm.

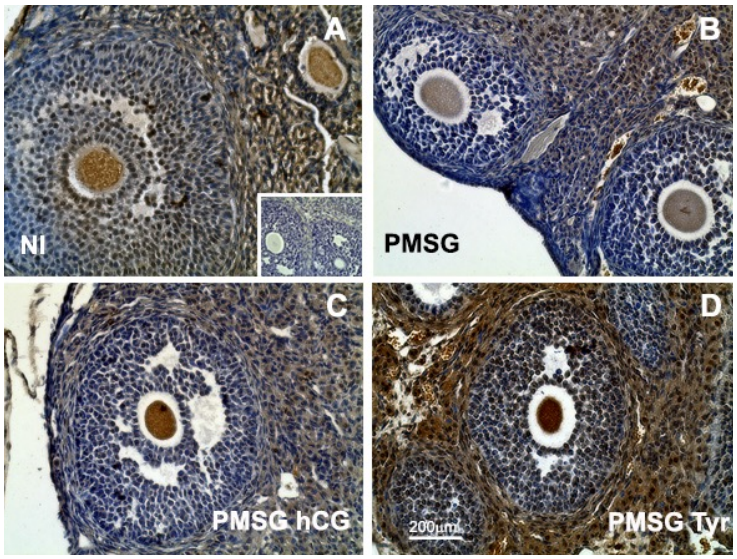

Figure 19. Histological Ovary Sections Co-Labeled with TAAR1. Histological sections of an ovary highlighting a tertiary follicle in non-injected (A), injected with PMSG (B) or PMSG hCG (C) or PMSG followed by treatment with tyramine (D). Localization of TAAR1 is found to be enriched not only around the follicle but at the oocyte and in the cumulus cells.

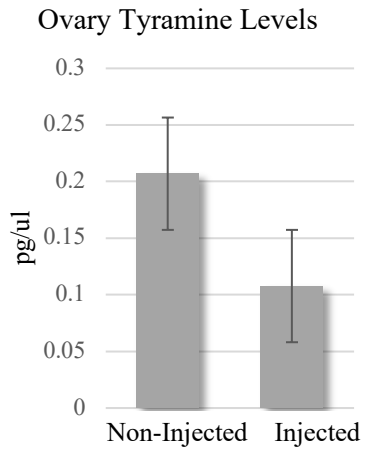

Figure 20. Quantification of Tyramine in Mouse Ovaries. Tyramine levels in the ovary were present in higher concentrations in mice that received no hormonal injections, compared to completely superovulated mice ($p=<0.05$, $n = 10$).

Discussion & Conclusion

The aim of my laboratory benchwork was to identify if the biogenic amine tyramine was present within the mouse ovary and explore its potential effect on the ovulatory process in mice. Previous research has shown that tyramine and its receptor TAAR1 are both present in uterine smooth muscle tissue in mice (Obayomi et al., 2017). It is also known that the cells of the theca externa that surround ovarian follicles share common morphological features with smooth muscle (Peck et al., 2019). This led to the formation of my hypothesis, that if tyramine was present within the mouse ovary, then female mice undergoing hormonal superovulation would exhibit heightened localization of both tyramine and TAAR1. The results of my study confirm the presence of tyramine within the mouse ovary and provide preliminary data suggesting that tyramine may have role in follicular maturation and ovulation. Further research into the actions of this monoamine may provide additional insight into understanding the signaling mechanisms involved in ovary function.

Standard protocols for superovulation in mice entail two intraperitoneal injections: PMSG and hCG. These sequential injections encourage follicular maturation (PMSG) and expulsion of oocytes out of their follicles during ovulation (hCG). Using immunohistochemistry, I observed a localization pattern of TAAR1 throughout the superovulation process. Non-injected ovaries showed no pattern of localization, however, after beginning ovulation induction with the first injection of PMSG, localization of TAAR1 appeared at the perimeter of the ovary, particularly along the membranous sac that surrounds the ovary, called the bursa. Localization was also observed within some of the activated follicles at the beginning of the maturation process (Figure 17B). After

addition of the second injection, hCG, localization of TAAR1 was observed in the corpus luteum of ruptured follicles, and along the perimeter of the ovary where oocytes were released during ovulation (Figure 17C). Ovaries that were not super-ovulated but were exposed to only tyramine *in vitro* appeared to have increased localization of TAAR1 within ovarian follicles. Localization of tyramine's receptor in non-injected ovaries exposed to tyramine *in vitro* resembled the pattern of localization of TAAR1 in ovaries undergoing hormonal superovulation, particularly the effect that PMSG had on TAAR1 localization (Figure 17D & Figure 18B).

To observe patterns of localization of tyramine's receptor further, I performed histological counter-staining to visualize oocyte-containing follicles at different stages of hormonal superovulation, and after exposure to tyramine. Similar to the observations made using immunohistochemistry, histologically prepared non-injected ovaries showed no definitive pattern (Figure 19A). After receiving the first injection of PMSG, TAAR1 was recruited to the perimeter of the tertiary follicles, which was even more prominent following injection of hCG (Figure 19B & 19C). The final experimental condition I analyzed using histology was PMSG with exposure to tyramine *in vitro*. The goal of this experimental condition was to observe if the localization of TAAR1 in PMSG + tyramine ovaries would resemble PMSG + hCG (standard superovulation). In this case, there was strong enrichment of TAAR1 in the theca externa of the follicle, the oocyte itself, and the cumulus cells that support the oocyte (Figure 19D). The pattern of localization between completely super-ovulated ovaries and PMSG + tyramine ovaries were similar. However, exposure to tyramine *in vitro* after injection of PMSG resulted in much stronger enrichment in those areas. The unique pattern of TAAR1 labeling observed through

immunohistochemistry and histology during different points of superovulation suggest that tyramine may be involved with cycling of oocytes during follicle maturation, due to the presence of tyramine's receptor within both the follicle and oocyte.

In the last set of experiments, I quantified the presence of tyramine in non-injected and completely super-ovulated (PMSG/hCG) ovaries using HPLC to determine if there was a relationship between levels of tyramine and ovulation. Determining a quantitative relationship between tyramine and ovulation would allow us to conclude how tyramine impacts cycling and ovulation of oocytes, or if there was no relationship between the two. After performing HPLC on these two experimental conditions, I noted that levels of tyramine were higher prior to ovulation and were reduced after. These HPLC results further confirm that tyramine does play a role in the ovulatory process. Performing additional trials using PMSG only, PMSG + tyramine, and non-injected + tyramine-treated ovaries would help specify when tyramine is being depleted. Understanding if tyramine is being depleted throughout superovulation, or only after ovulation will provide insight into whether tyramine has an ongoing role in both maturation and expulsion of follicles, or if tyramine's role is substantial during a brief period of superovulation.

Ultimately, the results of this study may provide foundational knowledge needed to begin exploring the role of tyramine in oocyte maturation and ovulation in humans. However, ovarian anatomy of *Mus Musculus* differs slightly from that of humans. In mice, a membranous sac called the bursa envelops the ovary and connects directly to the oviduct (Hillier, 2012). This membrane facilitates the movement of ovulated oocytes into the oviduct. The bursa has also demonstrated rapid accumulation and reabsorption of

fluid during ovulation in mice which suggests a possible role of the membrane controlling fluid homeostasis during ovulation and ovarian repair following ovulation (Zhang et al., 2013).

The bursa structure is not present in human ovarian anatomy. Rather, the movement of ovulated oocytes into the fallopian tube in humans is facilitated by fimbriae present at the distal end of the tube that "sweep" the oocyte through the fallopian tube towards the uterine body. The anatomical differences between mice and humans necessitate additional studies using both mouse and human tissue. In addition to conducting further experiments using mouse ovaries, performing identical trials using human ovarian tissue would determine the extent of tyramine's role in maturation and ovulation in humans. By better understanding the mechanisms of ovulation, this information could augment the existing protocols we use at the clinic for controlled ovarian stimulation, and even merit the development of new protocols to more effectively treat patients who are experiencing infertility related to ovulatory dysfunction.

Materials & Methods

Animals

Use of animals in this project was approved through the IACUC protocols 18-1606R and 20-1801R at Arizona State University (see Appendix B). All mouse tissues utilized for experiments were obtained from female C57BL/6J wild-type mice (Bar Harbor, ME) approximately eight weeks of age.

Superovulation & Tissue Procurement

For my experiments, I compared five different experimental conditions which are described in Table 9 below. These specific experimental conditions were utilized to

observe the additive effects of super-ovulatory injections and to establish baseline values that I could compare to conditions involving the addition of tyramine.

I followed standard protocols for superovulation for my study, which involved a regimen of pregnant mare serum gonadotropin [PMSG] and human chorionic gonadotropin [hCG] injections (The Jackson Laboratory, 1998). Female mice undergoing partial or complete superovulation were treated using standard intraperitoneal [IP] injection protocols, illustrated in Figure 21. An initial IP injection of 5 IU of PMSG was administered, followed 48 hours later with an IP injection of 5 IU of hCG (The Jackson Laboratory, 1998). Approximately 14 hours after hCG was administered, completely super-ovulated mice were anesthetized using isoflurane and euthanized through cervical dislocation. Following cervical dislocation, the mouse is prepped for dissection by applying 70% ethyl alcohol to the abdomen. To remove the ovary, I made a midline incision down the ventral side of the mouse, starting below the ribcage down to the urethral orifice. Once the body cavity was opened, the fat pad is lifted away exposing the reproductive system. The ovary can be identified proximally to the oviduct and uterine horn.

Whole ovaries for experimental conditions that did not utilize tyramine were immediately placed in a fixative solution of 4% paraformaldehyde made with phosphate-buffered saline [PBS] and left to fix overnight at 4ºC to stabilize and preserve the tissue. For experimental conditions that underwent tyramine exposure: non-injected + tyramine & partial superovulation + tyramine, I warmed KSOM medium to approximately 37º, and added a physiological dose of 1nM tyramine to the solution. The ovaries were incubated in the tyramine-containing media for 45 minutes, and once the exposure time was

complete, they were removed from the media and fixed using 4% paraformaldehyde solution and left to fix overnight at 4ºC.

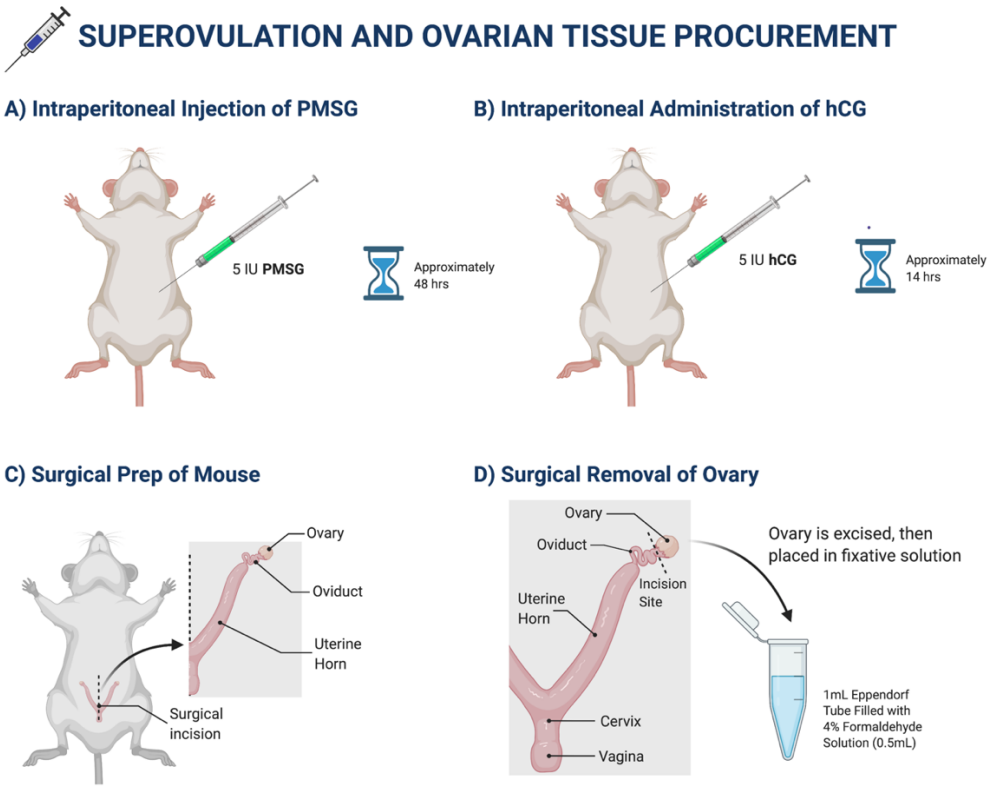

Figure 21. Superovulation and Ovarian Tissue Procurement. Created with BioRender.com

Immunohistochemistry of Frozen Ovarian Tissue Sections

After fixing isolated ovarian tissue in 4% paraformaldehyde, I prepared samples from each experimental condition for immunohistochemistry using antibodies that specifically bind Tyramine and its receptor: Trace Amine Associated Receptor 1 [TAAR1]. The fixative solution was removed and replaced it with 30% sucrose solution.

The tissues were incubated in the sucrose solution overnight at 4ºC. The next day, the tissue are positioned in molds filled with Tissue-Tek O.C.T. (VWR). The molds were lowered into 2-propanol that was cooled with liquid-nitrogen until the Tissue-Tek had solidified. The samples were stored in a -80ºC freezer. I sectioned each block at 40µm using the Leica CM1950 Cryostat onto charged slides before labeling the samples for immunohistochemistry.

To prepare for labeling, I drew a hydrophobic barrier around the perimeter of each slide containing ovarian sections (see Figure 22). This barrier prevented solutions from running off the slide during each step of the immunohistochemistry protocol.

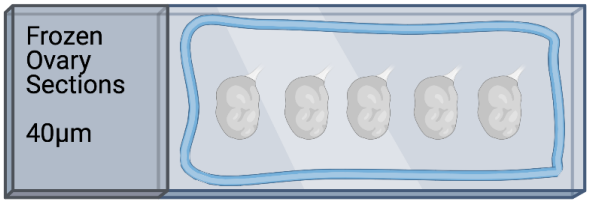

Figure 22. Example Slide of Ovarian Sections. Five sections of ovarian tissue are present on the slide, with the hydrophobic barrier around the perimeter in blue. Created with BioRender.com.

I blocked and permeabilized the ovary sections prior to labeling. Permeabilizing solution was made using 2% formaldehyde and 1% Tween-20 in 1x PBS. I incubated the tissues in permeabilizing solution overnight at 4ºC, which created spaces in the membrane so antibodies could bind inside the cells of the tissue for labeling. The following day, I discarded the permeabilizing solution and rinsed the slides in buffer

containing 1% Bovine Serum Albumin [BSA] diluted in PBS, or PBS/BSA three times for five minutes each. After the tissues were rinsed completely, I added primary antibody to the tissues to begin the immunolabeling process. Rabbit anti-TAAR1 primary antibody (ThermoFisher, OSR00119W) was added at approximately 1:1000 in PBS/BSA solution and incubated overnight at 4ºC. Following incubation in the primary antibody, I rinsed the tissues in PBS/BSA buffer three times for five minutes each, to flush away any residual primary antibody.

Next, I added light-sensitive secondary antibodies fused to fluorophores to the tissue for fluorescent visualization. Anti-rabbit Alexa 488 (ThermoFisher Scientific, 1:1000 in PBS/BSA) was added as the secondary antibody to the anti-TAAR1. Phalloidin (ThermoFisher, A12380) was also added into the secondary antibody solution—a toxin from the Amanita phalloides mushroom that selectively binds F-actin and is conjugated to Alexa 568. I wrapped the tissues in foil and incubated them overnight at 4ºC. The following day, I rinsed the tissues three times for five minutes each in 1x PBS and sealed each slide with Vectashield and a coverslip for imaging. Scans of the ovary sections were taken as a set of tile images using a Leica SP5 confocal microscope, housed in the Keck Bioimaging Lab at Arizona State University, with a 20x objective at a depth of 20µm in 1µm steps.

Paraffin-Embedded Histology

Mouse ovaries from each experimental condition were obtained as described above and fixed in 4% paraformaldehyde overnight at 4ºC. Following fixation, I rinsed the tissues using 1x PBS to remove all fixative solution. I repeated this process three times before beginning the dehydration process for rapid dehydration and paraffin

embedding. To dehydrate the tissue, I moved the tissues through increasing concentrations of ethanol: 70%, 80%, 90%, 95%, 100% (twice), before moving into a 50/50 solution of ethanol/ClearRite (Thermo Scientific), 100% ClearRite (twice), then ClearRite/Paraffin and finally, into 100% paraffin wax. After reaching 100% paraffin wax, I placed the tissues in embedding molds to allow the paraffin to solidify completely before I sectioned them.

I cut the embedded ovaries into 5µm sections using the Leica RM1950 microtome and collected the sections onto charged microscope slides. I cleared the slides of excess wax using ClearRite and rehydrated the tissues using the series of steps outlined in Figure 23.

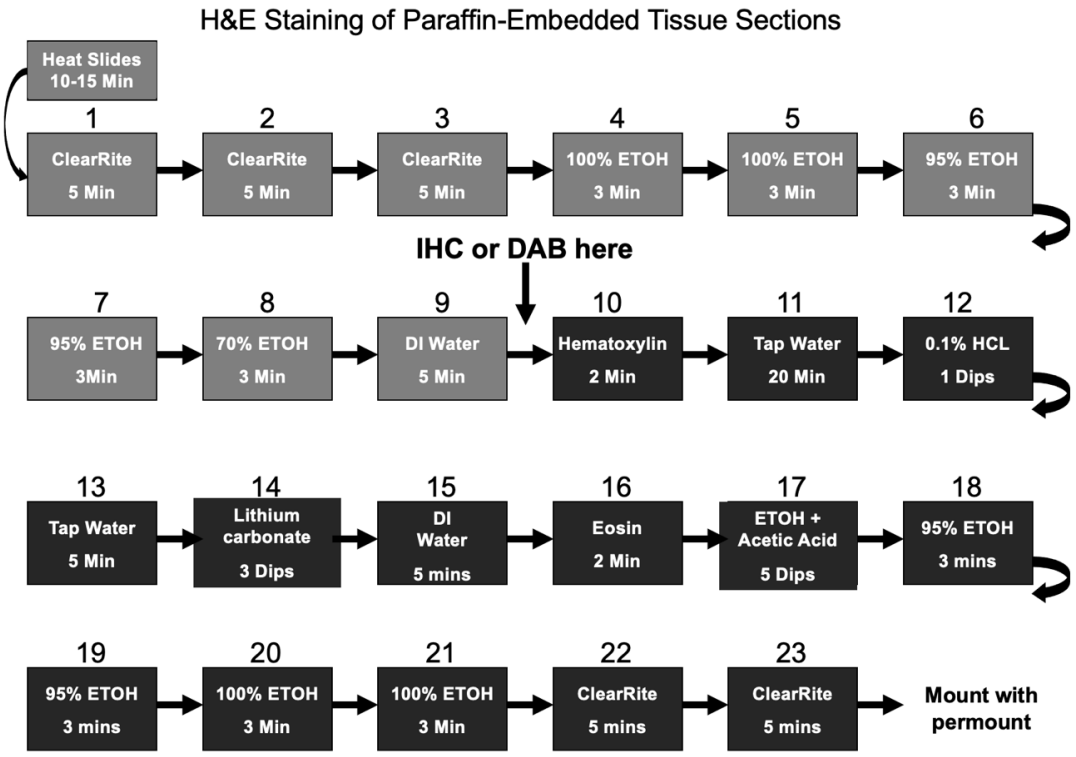

Figure 23. H&E Staining of Paraffin-Embedded Tissue Sections. Credit: Shelbi Peck 2017.

After completion of step 9 in deionized water, I incubated my ovarian sections in rabbit anti-TAAR1 antibody (ThermoFisher; 1:1000) overnight in a humidified chamber. Following the incubation period, I rinsed my slides three times for five minutes each using PBS/BSA solution before adding anti-rabbit horseradish peroxidase (Pierce; 1:500) and incubated the slides in the humidified chamber overnight. The following day, I rinsed the slides again and added 3,3'-Diaminobenzidine (VectorLabs), or "DAB" for ten minutes. DAB quickly oxidizes in the presence of horseradish peroxidase and produces a visible brown precipitate. Once a visible brown precipitate had formed, I rinsed the slides thoroughly with Barnstead water to prevent excessive staining. I proceeded through the remaining steps of the H&E staining procedure in Figure 21 starting at step 10 with the hematoxylin counterstain and ending with the slides being sealed using permount. Once the slides had dried completely, I visualized the slides using the ThermoFisher Scientific Evos FL auto live cell imaging system, housed in the WM Keck Bioimaging Laboratory at Arizona State University.

High-Performance Liquid Chromatography

High-Performance Liquid Chromatography [HPLC] is a quantitative technique that identifies and quantifies the components of a mixture by separating out each element as it flows through a pressurized column. Mouse ovaries from non-injected and completely super-ovulated experimental conditions were obtained as described above. Immediately following dissection of the ovaries, I flash-froze the tissues in 1.5mL Eppendorf tubes and stored at -80ºC until I analyzed them using HPLC. The first step of preparation for HPLC is to digest the tissues using a perchloric acid solution. I added each tissue to a small Eppendorf tube containing perchloric acid and left them in an ice

bath for a total of thirty minutes to facilitate disruption of the tissue. Next, I pulverized the partially dissolved tissue using an Eppendorf tissue grinder and pestle, before centrifuging the tubes for ten minutes to ensure that any remaining pieces of tissue that had not dissolved formed a pellet at the bottom of the tube. Centrifuging the tubes to separate out undissolved tissue reduced the likelihood that pieces of tissue would block the lines of the HPLC system during analysis.

Using a micropipette, I loaded 10µL of each sample into the ESA CoulArray HPLC system. The system generated characteristic chromatographic peaks for the amines that flowed through the column, which I compared between non-injected ovarian samples and completely super-ovulated ovarian samples. The U.S. Department of Agriculture's Arid-Land Agricultural Research Center in Maricopa, AZ graciously allowed for temporary use of the ESA CoulArray HPLC System for this project.

Statistical analyses

Quantification of the HPLC samples were obtained from 10 mice. Unless indicated otherwise results are shown as the mean ± SD. Samples that passed the normal distribution test were analyzed by a student's t test and were considered significantly different if $p < 0.05$.

CHAPTER 8

CONCLUSION

Reproductive medicine is a specialized branch of medicine that aims to diagnose and treat patients who are experiencing reproductive dysfunction. In Chapter 1 & 2 of my dissertation, I described normal reproductive function to establish a standard for how reproduction works under the best of circumstances. This provided the necessary framework for addressing the first aim of my dissertation, to *describe how the reproductive system works in order to diagnose and treat reproductive disorders*. Through decades of research, numerous pathologies of infertility have been uncovered that span anatomical, physiological, and genetic origins. Both men and women can experience reproductive dysfunction which impacts their ability to conceive naturally.

During my time working at the fertility clinic, I noticed patients coming to the clinic with various forms of infertility, and some who were unsure why they were unable to conceive on their own. Using the knowledge and experience I attained through working at the clinic, I characterize these forms of reproductive dysfunction in Chapter 3: Diagnosis and Characterization of Reduced Fertility. In men, reduced fertility typically manifests as either defective spermatogenesis, or problems with the transport of sperm through the reproductive tract (NICHD, 2017). For women, reproductive dysfunction falls into one of four categories: ovarian, fallopian tube, uterine, or cervical infertility. Within each of these broad categories there are anatomical, endocrine, and genetic factors that can individually impact a woman's ability to become pregnant and carry a pregnancy to term.

Many patients who have received a concrete diagnosis for their reproductive dysfunction have sought treatment at a fertility clinic. At fertility clinics such as the one where I worked, we can attempt to address a patient's infertility using treatment options such as timed intercourse, ovulation induction, and Assisted Reproductive Technologies [ARTs] like intrauterine insemination, *in vitro* fertilization, and intracytoplasmic sperm injection. This brings us to the second aim of my dissertation, to *examine the development and use of fertility treatments and ARTs in reproductive medicine*. In Chapter 4: Fertility Treatments and Assisted Reproductive Technologies, I began by describing the history of how the treatments utilized at fertility clinics were developed. Then, using the unique perspective I have from working in the field of reproductive medicine, I describe why a patient would use each treatment, treatment effectiveness, and discuss protocols I performed within the fertility clinic laboratory.

I wrote the first four chapters of my dissertation with patients in mind--specifically, to serve as a resource for individuals experiencing reduced fertility. These chapters provide foundational information for patients who want to learn more about reproductive health, understand what treatments are offered at a fertility clinic and their associated costs, and how laboratory staff work with patient samples to help individuals overcome their reduced fertility. As a member of the laboratory staff, I noticed that many patients were interested in what went on in the lab, but we had very little time to explain all the details of the kind of work we did. The information available online for patients to learn more about the inner workings of a fertility clinic laboratory is not very detailed. Clinic websites typically describe the patient's role and how the treatment process works for the patient (diagnostics, medicated ovulation induction, retrieval, embryo transfer,

etc.), but leave out information on how the laboratory specifically facilitates the reproductive process. Therefore, I wanted to help bridge the gap that existed between publicly available resources for patients seeking fertility treatment and the intricate work of fertility clinic laboratory staff.

Across the globe, millions of babies have been born through fertility treatments and ARTs provided by fertility clinics. However, implementation of these techniques and technologies in the field has had unanticipated ethical repercussions. As a field, reproductive medicine has not been regulated effectively in the United States. Lack of sufficient regulatory oversight reveals loopholes that can lead to misguidance, discrimination and even exploitation of patients and donors. This led to the development of the third aim of my dissertation, *to analyze prominent ethical issues I observed in the field of reproductive medicine*. In Chapter 5: Ethical Dilemmas in Fertility, I examined three prominent ethical quandaries that I observed while working at the fertility clinic: profiling and anonymity of sperm donors, oocyte donor compensation, and the ramifications of specialized forms of pre-implantation genetic testing [PGT]. I discussed how these issues arose at the clinic where I worked, why each constitutes as an ethical issue, and the gaps in regulations and legislation that led to these issues.

The three ethical dilemmas I chose to discuss are by no means the <u>only</u> ethical issues in fertility medicine; however, these were the prominent issues I observed during my time at the clinic. Our ability to manipulate the reproductive process has surpassed the current regulations in place that protect patients and the children born using ARTs, which allows these ethical dilemmas to arise. Ultimately, I call for the adoption of new policies, procedures, restrictions and sanctions against clinics and sperm/oocyte banks

that do not comply. Establishing a more rigid regulatory framework for fertility medicine will help to protect the best interests of patients, donors, fertility clinics, and sperm/oocyte banks alike.

Despite everything we know about reduced fertility, in over 30% of cases, physicians are unable to pinpoint a reason for an individual's reduced fertility, which leads to a diagnosis of idiopathic infertility (Chandra et. al, 2013; Sadeghi, 2015). As ARTs become a more prevalent method for conception, it is crucial to continue developing more effective methods to diagnose and treat infertility. This led to the development of the final aim of my dissertation, to *review novel methodologies that may advance the field of reproductive medicine*. Research helps to fill in the gaps present in current knowledge of reproductive physiology and allow for the continual advancement of fertility medicine. During my time working at the clinic, I became aware of some specific pathologies of infertility that we were unable to treat, and where technological limitations could be improved to make our workflow in the lab smoother. In Chapter 6, I covered current and future research in the field of fertility medicine. This included novel therapies and remote fertility testing options for both female and male patients, as well as the use of artificial intelligence in the embryology laboratory. For each area of research, I gave personal insight into the ways these therapies and technologies could be implemented at my clinic and who would benefit from them the most.

The studies described in Chapter 6 had all progressed to implementation in human clinical trials. However, before any protocol can be implemented in humans, numerous phases of clinical and pre-clinical trials are conducted to ensure safety and efficacy of new techniques (Faggion, 2015). The final chapter covers an animal trial that I conducted

to explore the role of the biogenic amine tyramine on the process of ovulation in mice. The results of my study suggest that tyramine may influence maturation and ovulation of oocytes from the ovary, resembling the effect of standard superovulation protocols. This preliminary study raises fundamental questions regarding the role that tyramine may play in the human reproductive system and demonstrated that there are other molecules that interact with reproductive function that have not been taken into consideration previously. If tyramine exhibits similar effects on oocyte maturation and ovulation in humans, this may lead to the development of shortened protocols used for ovarian stimulation, which has implications in both agriculture and human medicine.

ARTs have revolutionized the way infertility is perceived from a clinical standpoint. Once thought of as an insurmountable burden for women alone, infertility is now recognized as both a man and woman's issue and, in most cases, can be more successfully treated now than ever before. A diagnosis of reduced fertility or infertility presents a mere hurdle to procreation rather than a complete termination of a couple's aspirations to start a family. Fertility clinics have become beacons of hope for individuals who are struggling to overcome infertility by providing treatments and ART services to patients. My dissertation has accomplished each of its intended goals, utilizing a combination of literature review, clinical experience, and laboratory research. It is my hope that through personal experience working at a fertility clinic and conducting original research have generated a unique perspective to provide a much-needed resource to patients seeking fertility treatment.

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APPENDIX A

TABLES

Table 1

Relevant Hormones & Molecules in the Male Reproductive System

Molecule	Site of Production	Role within the Reproductive System
Gonadotropin-Releasing Hormone [GnRH]	Hypothalamus	GnRH levels are low prior to the onset of puberty but is responsible for the later production of FSH and LH.
Follicle-Stimulating Hormone [FSH]	Anterior Pituitary	FSH is essential for the initiation of the first meiotic division of primary spermatocytes, as well as the sequestering of testosterone for maintenance of spermatogenesis.
Luteinizing Hormone [LH]	Anterior Pituitary	LH works in conjunction with FSH to promote both synthesis and secretion of testosterone in Leydig cells.
Testosterone	Leydig cells within the Testes primarily and adrenal gland	Testosterone plays numerous roles in the male reproductive system. Initially, testosterone is essential for development of male reproductive anatomy. Following puberty, testosterone plays an active role in libido and the maintenance of spermatogenesis. Presence of testosterone negatively regulates production of FSH and LH by the pituitary.
Estradiol	Leydig cells, fat, liver, adrenal glands	Present in much smaller quantities in men than in women, estradiol prevents premature apoptosis of spermatozoa.
Activin & Inhibin	Sertoli cells	Activin and Inhibit help to regulate sperm count by increasing (activin) or decreasing (inhibin) levels of FSH.

Note. The information provided in Table 1 is an adaption of information from *Anatomy and Physiology* published by Betts et al. in 2013. I also conducted blood serum immunoassays using the TOSOH AIA900 system at the clinic on male fertility hormones, although these hormones and molecules were not assessed as frequently as female hormones were.

Table 2

Relevant Hormones in the Female Reproductive System

Molecule	Site of Production	Role within the Reproductive System
Gonadotropin-Releasing Hormone [GnRH]	Hypothalamus	GnRH levels are low prior to the onset of puberty but is responsible for the later production of FSH and LH.
Follicle-Stimulating Hormone [FSH]	Anterior Pituitary	Stimulates activation of multiple primordial follicles in the ovary. FSH production is suppressed by secretion of estrogen from follicles.
Luteinizing Hormone [LH]	Anterior Pituitary	Stimulates the rupture of a tertiary follicle, leading to ovulation. Luteinization of theca cells post-ovulation induces formation of the corpus luteum.
Estrogen	Adrenal glands, ovaries, fat tissue	Promotes development of secondary sex characteristics. Tertiary follicles produce high amounts of estrogen as a part of a negative feedback loop in relation to FSH and LH, causing only the dominant follicle to survive. Low levels of estradiol promote shedding and production of a new uterine lining.
Progesterone	Corpus luteum of ovaries	Acts as a signal to the uterus to initiate thickening of the uterine lining to prepare for embryo implantation, or initiates shedding of the uterine lining if no implantation occurs.
Human Chorionic Gonadotropin [hCG]	Embryo	Produced during implantation. Urine Pregnancy Tests are commonly used to qualitatively detect hCG as an indication of the establishment of a pregnancy.

Note. The information provided in Table 2 is an adaption of information from *Anatomy and Physiology* published by Betts et al. in 2013. I also drew upon my clinical fieldwork to generate this table, largely from experience conducting blood serum immunoassays on the above hormones and molecules using the TOSOH AIA900 system. Female fertility hormone assays were performed most often in the lab, with upwards of 150-200 samples analyzed in a single day during the busy weeks of patient cycles.

Table 3

Macroscopic Semen Analysis Parameters

Parameter	Variations	Potential Clinical Significance
Liquefaction (@Room Temperature)	15-30 minutes	Normal
	30-60 minutes	Normal but less ideal
	60+ minutes	Abnormal; prostatic dysfunction
Appearance	Grey	Normal
	Clear	Low sperm count which can be due to numerous medical, environmental or lifestyle choices such as varicocele, infection, anabolic steroid usage, exposure to radiation, smoking/drinking habits, and weight.
	Red/Brown	Red blood cells in ejaculate (hemospermia) which can change pH of semen. Possible urinary tract infection or STI.
	Yellow	Urine in semen or can be a result of vitamins/drugs (WHO, 2010).
Volume	Low (<1.5mL)	Ejaculatory duct obstruction, or loss of sample during collection (hypospermia)
	High (<5.5mL)	Inflammation of accessory glands (hyperspermia)
Viscosity	1+ / Slight stringing	Normal
	2+ / Moderate stringing	Normal
	3+ / 4+	Abnormal; Semen hyperviscosity which can lead to decreased sperm motility.
pH	< 7.0	Potential ejaculatory duct obstruction, blockage/poor development of seminal vesicles, or congenital bilateral absence of vas deferens (WHO, 2010).
	7.1-8.0	Normal range (Banjoko & Adeseolu, 2013)
	>8.0	Infection

Note. The information provided in Table 3 is an adaption of information from the *World Health Organization's laboratory manual for the Evaluation and processing of human semen: 5th Edition* and Banjoko & Adeseolu, 2013. I also drew upon personal experience from my time at the clinic where on average, I conducted ~10 semen analyses each day using the same criteria provided by the WHO. Macroscopic parameters were always observed first, followed by analysis of microscopic parameters described in Table 4.

Table 4

Microscopic Semen Analysis Parameters

Parameter	Variations	Potential Clinical Significance
Motility	Progressive	Normal
	Non-Progressive	Abnormal. This condition is also called *asthenozoospermia*, which can be a result of metabolic deficiencies or abnormal tail shape of sperm. There is also a correlation between asthenozoospermia and increased DNA fragmentation of sperm (Ortega et al., 2011)
	Immotile	Abnormal. Caused by the same factors as non-progressive motility, except the majority of sperm are not moving—referred to as *necrozoospermia*.
Concentration	<14.99 x 10^6 per mL	Low sperm concentration is referred to as *oligozoospermia*. There are three grades of oligozoospermia: mild, moderate, and severe. This condition can occur due to an obstruction, infection, Y chromosome abnormality, androgen deficiency, or from drug/alcohol usage.
	>15 x 10^6 per mL	Normal
Sperm Count	No sperm present	Abnormal. *Azoospermia*. Repeat semen analysis is required to confirm azoospermia. A follow up with a urologist may be needed to determine the exact cause of the condition.
	0 < count < 38.99 x 10^6 per ejaculate	Abnormal. Related: oligozoospermia.
	>39 x 10^6 per ejaculate	Normal
Total Motile Sperm Count	<39.99%	Abnormal—see asthenozoospermia and/or necrozoospermia.
	>40%	Normal

Parameter	Variations	Potential Clinical Significance
Agglutination (head-to-head, tail-to-tail or mixed clusters)	None	Normal
	Slight/Isolated: <9.99 spermatozoa Moderate: 10-49.99 Large: >50 Gross: All interconnected	Any degree of agglutination is abnormal—the more severe the agglutination is, the more this impacts male fertility. Sperm that are stuck together are unable to travel through the female reproductive system. Causes: bacterial infection, inflammation of accessory glands, high concentration.
Aggregation (clumping of sperm and debris to mucus strands)	None	Normal
	Few Many	By itself, this parameter is not a huge cause for concern, however if it is seen in tandem with agglutination, this may be due to the same underlying issues.
Round Cells (spermatids, spermatocytes, or leukocytes)	None	Normal
	<1 million per mL	Less ideal but normal
	>1 million per mL	Abnormal; *pyospermia*/*leukocytospermia*. Potential inflammation, infection, varicocele, or STI.
Morphology (Typically given an overall score calculated as the # of normal sperm/100 observed)	<4% normal forms	Sperm may have poor motility or experience other deformities that render them incapable of reaching or fertilizing an oocyte. Some of these deformities include: Head defects: tapered, pyriform, round, or amorphous, multiple heads, or heads with >20% occupied by vacuoles. Acrosomal defects: No acrosome or very small acrosome. Neck and Midpiece: Bent or asymmetrical neck, too thick or thin midpiece. Tail defects: Short, bent, coiled, multiple tails.
	>4% normal forms	Normal

Table 4

Note. The information provided in Table 4 is an adaption of information from the *World Health Organization's laboratory manual for the Evaluation and processing of human semen: 5th Edition*; Ortega et al., 2011. I also drew upon personal experience from my time at the clinic, where on average I conducted ~10 semen analyses each day using the same criteria provided by the WHO. In a typical semen analysis, microscopic parameters are observed after the microscopic parameters, with a small aliquot of the sample observed in a Makler Counting Chamber.

Table 5

Fertility Blood Serum Assay Ranges by Cycle Day

Molecule	Cycle Day (if applicable)	Result
Estradiol	Day 3	Normal range: 25-200pg/mL. Abnormally high estradiol levels may indicate diminished ovarian reserve/primary ovarian insufficiency, or presence of a functional ovarian cyst. Postmenopausal estradiol is <10pg/mL (Mayo Clinic Laboratories, n.d.-b)
Progesterone	Day 3	Day 3 corresponds to the follicular phase when progesterone is low. Normal range: < or = 0.89ng/mL (Mayo Clinic Laboratories, n.d.-c)
	Day 21	Following ovulation and entrance into the luteal stage, progesterone levels increase which is used as confirmation of ovulation and increases throughout pregnancy. Normal range: 1.8-24 ng/mL (Mayo Clinic Laboratories, n.d.-c).
Follicle-Stimulating Hormone [FSH]	Day 3	Normal range: 5-20 mIU/mL. For FSH values that are < or = 10 mIU/mL, this may indicate that the patient will respond poorly to ovarian stimulation medications (Broekmans et al., 2006).
Luteinizing Hormone [LH]	Day 3	Normal range: 1.9-14.6iU/L. During the follicular phase, LH is low and follows a similar pattern as FSH. A mid-cycle surge of LH triggers ovulation within 48 hrs (Mayo Clinic Laboratories, n.d.-d)
Prolactin	Day 3	Normally, prolactin levels are between 4.8-23.3ng/mL (Mayo Clinic Laboratories, n.d.-e). In cases of hyperprolactinemia where levels of prolactin are high, this may lead to anovulatory cycles and subfertility.
Anti-Mullerian Hormone [AMH]	N/A	In reproductive age women: 0.9-9.5 ng/mL is considered normal. Low AMH may indicate poor response to stimulation using gonadotropins (Fritz & Speroff, 2011).
Thyroid Stimulating Hormone [TSH]	Day 3	Normal levels of TSH in the blood stream range from 0.3-4.2mIU/L in women > or = 20 years of age. (Mayo Clinic Laboratories, n.d.-f). There is an inverse relationship between levels of TSH and free T4—if TSH levels are high, this indicates hypothyroidism where the thyroid gland is producing more TSH to compensate for a deficiency in thyroid hormones. When TSH levels are low, this indicates hyperthyroidism—the thyroid gland is overproducing hormones which suppresses production of TSH.

Molecule	Cycle Day (if applicable)	Result
Free and Total [T4] (Thyroxine)	Day 3	Free and total T4 measurements show how thyroid hormones are produced and utilized by the body. In reproductive age women, the normal range of Total T4 is 5.9-13.2 mcg/dL, and Free T4 between 1.0-1.6ng/dL (Mayo Clinic Laboratories, n.d.-g). T4 is synthesized from TSH, so these tests are ordered together to show a more complete picture of how thyroid hormones are cycled. Low levels of Free and/or total T4 indicate hypothyroidism. This can lead to development of ovarian cysts and increase production of prolactin in the body (see Prolactin). Conversely, hyperthyroidism can cause high levels of Free/Total T4, which has been linked to increased miscarriage.
Human Chorionic Gonadotropin (hCG)	N/A	A value of >50 mIU/mL observed at least 3 weeks since the patients last menstrual period indicates the patient is pregnant. Less than 50 mIU/mL indicates no pregnancy, miscarriage, or ectopic pregnancy.

Note: The numerical values listed in Table 5 were collected using Mayo Clinic Laboratory's test catalog (Mayo Clinic Laboratories, n.d.-a; Cited as Mayo Clinic Laboratories, n.d.-b-g). The clinical interpretations and applications described in this table were adaptations of information from publications by Broekmans et al. (2006), Fritz & Speroff (2011), as well as from my work in the clinic where I conducted blood serum immunoassays on the above hormones and molecules using the TOSOH AIA900 system and charted patient's results in our electronic health record system.

Table 6

Breakdown of IUI/IVF Costs

Aspect of Treatment	Cost
Stimulation Medications	$200-$5,000 depending on stim protocol
Ultrasounds/Bloodwork	$1,000-$3,000
Sperm Prep + Insemination, IUI only	$600-1,500
Retrieval Procedure + Anesthesia IVF only	$300-$1,500
ICSI IVF only	$500-$2,000
Sperm/Oocyte Donor Use	Sperm: $500-$1,000
	Oocyte: $10,000-$20,000
Genetic Testing/Embryo Screening IVF only	$2,000-$7,000
	*Varies by company, type of testing, # of embryos tested
Frozen Embryo Transfer (FET) IVF only	$700-$6,800
	*First FET is often included
Surrogacy IVF only	$50,000-$150,000
Embryo Storage IVF only	$350-$1,200 (annually)

Note. Ranges provided in Table 6 are estimates based upon observations made using online fertility clinic price charts in 2020 across the U.S., as well as billing references from the clinic where I worked. Prices for fertility treatments are not presented in the same format across clinics—some itemize costs, while others present their prices as packages that include multiple items on this list. The values presented in this table are estimates for itemized aspects of receiving fertility treatment.

Table 7

Common Medications Used in Fertility Medicine

Stage of Cycle	Medication(s)	Function
Suppression (Also called "Down Regulation")	Oral Contraceptive Pill [OCP]: Desogen	OCP's use progesterone or a combination of both estrogen and progesterone to suppress follicular development and inhibit ovulation.
	GnRH Agonist: Lupron	GnRH agonists induce production of GnRH by the hypothalamus. This suppresses production of FSH and LH from the pituitary gland and prevents ovulation.
	GnRH Antagonist: Cetrotide/Ganirelix	GnRH antagonists regulate production of FSH/LH to prevent the LH surge that occurs prior to ovulation.
Stimulation	Progonadotropin: Clomiphene Citrate (Clomid), Serophene	Progonadotropins are estrogen-blocking medication that promotes the release of GnRH so FSH and LH production begins. In men: Clomiphene is prescribed to help stimulate production of FSH and LH to aid spermatogenesis for men with low sperm count (Earl & Kim, 2019).
	Aromatase Inhibitor: Femara, Letrozole	Femara and Letrozole are orally administered aromatase inhibitors that lower the production of estrogen, which causes the body to begin producing more FSH/LH to compensate.
	Human Menopausal Gonadotropin [hMG]: Menopur, Repronex, Pergonal, Humegon	hMG medications contain FSH and LH, which provides a continual supply of both essential hormones needed for growth of ovarian follicles. In men: hMG is used to encourage sperm production in men with reduced fertility related to hypogonadism
	FSH: Gonal-F, Follistim, Bravelle, Fertinex	FSH injections supplement the active role that naturally produced FSH plays in promoting development of dominant follicles. These medications can also be used to treat FSH deficiency.
	LH: Luveris	LH injections can be prescribed in tandem with FSH injections to stimulate production of follicles. These medications can also be used to treat LH deficiency.

Stage of Cycle	Medication(s)	Function
Trigger	hCG: Pregnyl, Novarel, Ovidrel, Profasi	hCG is used to trigger ovulation at the end of a stimulation cycle. In men: hCG is used to encourage sperm production in men with reduced fertility related to hypogonadism
Other: Post-OI	Progesterone suppositories	Progesterone suppositories assist with the thickening of the uterine lining and embryo implantation process.

Note. Medication information provided in Table 7 came from experience garnered during my time working at the clinic. This was primarily from communications with the licensed practical nurses and registered nurses at the clinic who were responsible for ordering medications and providing "med teach" lessons to instruct patients on how and when to take each medication during their cycle. Hormone dosage determination is typically determined using a resource such as the MicroMedex.

Table 8

Types of Pre-Implantation Genetic Testing

Test Name	What the test analyzes	Who would use this type of test
PGT-A	Sex selection Aneuploidy: Abnormal number of chromosomes	Patients who would like to know the sex of their embryos for family planning, experienced recurrent pregnancy loss or repeated IVF cycle failures, those who have a child with a genetic disorder, or in cases of advanced maternal age.
PGT-M	Monogenic disorders: Disorders caused by an abnormality in a single gene.	Couples with one or more partners that have or carry a single gene disorder such as muscular dystrophy, cystic fibrosis, Huntington's Disease, etc.
PGT-SR	Structural Rearrangements: Complex genetic disorders due to translocation or inversion of genetic material on a chromosome	Patients who have or are carriers for a chromosomal inversion, reciprocal translocation or Robertsonian translocation.

Note. Information for Table 8 was compiled using information provided by Cooper Surgical Fertility solutions regarding the types of PGT they offer to clients, and what the tests screen for specifically (Cooper Surgical, 2018).

Table 9

Role of Tyramine in the Mouse Ovary: Experimental Conditions

Name	Stimulation Protocol
Non-Injected (NI)	Unstimulated
Non-Injected (NI) + Tyramine	Tyramine *in vitro* only
Partial Superovulation	PMSG only
Partial Superovulation + Tyramine	PMSG + Tyramine *in vitro*
Completely Superovulated	PMSG + hCG

Note. Experimental conditions used during experiments. PMSG = Pregnant Mare Serum Gonadotropin. hCG = Human Chorionic Gonadotropin.

APPENDIX B

APPROVED IACUC PROTOCOLS FOR TYRAMINE STUDY

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 312
Tempe, Arizona 85287-6111
Phone: (480) 965-4387 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number:	**20-1801R**
Protocol Title:	**The Role of Tyramine in the Mouse Reproductive System**
Principal Investigator:	**D. Page Baluch**
Date of Action:	**7/1/2020**

The animal protocol review was considered by the Committee and the following decisions were made:

The protocol was approved by Designated Review as presented.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree.

Total # of Animals:	**1,040**	
Species:	**Mice**	**Unalleviated pain/distress: No**
Protocol Approval Period:	**7/1/2020 – 6/30/2023**	
Sponsor:	**N/A**	
ASU Proposal/Award #:	**N/A**	
Title:	**N/A**	

Signature: Nicole Shepherd for N.Henderson Date: 7/2/2020
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 312
Tempe, Arizona 85287-6111
Phone: (480) 965-6788 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number:	**18-1606R RFC 1**
Protocol Title:	**Role of Tyramine in the Mouse Reproductive System**
Principal Investigator:	**Page Baluch**
Date of Action:	**12/5/2017**

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was approved to add an additional location to the protocol.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree.

Total # of Animals:	**1,334**	
Species:	**Mice**	**Unalleviated Pain/Distress: No**
Protocol Approval Period:	**8/3/2017 – 8/2/2020**	
Sponsor:	**National Institute of Health**	
ASU Proposal/Award #:	**TBD**	
Title:	**Hormonal Modulation Studies**	

Signature: _____ for C. Miller Date: 12/8/2017
IACUC Chair or ***Designee***

Cc: IACUC Office
IACUC Chair