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CRISPR Genetic Editing: Paths for Christian Acceptance and Analysis of In Vivo and In Vitro Efficiency

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Paths for Christian Acceptance and Analysis of *In Vivo* and *In Vitro* Efficiency

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ABSTRACT

With advancements in CRISPR-cas9 broadening the potential paths for clinical usage of genetic editing, conversations about genetic editing have grown to outside simply scientific communities and into mainstream conversations. This study focuses specifically on Christian discourse of genetic editing and locates four major tensions for many Christians when they think about genetic editing: beginning of life, Creator-human relationship, imago Dei, and stewardship. With these major concerns in mind, I identify epigenetics, somatic cell genetic editing, and *in vivo* genetic editing research as important research paths to pursue as they can potentially produce techniques that more Christian individuals would feel comfortable using. I pursue one of these paths and conclude with an experimental proposal for an analysis of *in vivo* and *in vitro* CIRSPR-Cas9 efficiency in regards to on- and off-target rates.

INTRODUCTION

Not only is genetic editing and its rapid advancements a topic within the scientific community, it has been incorporated into mainstream conversations. Specifically, the discovery of and recent enhancements of the genetic editing technique, CRISPR-cas9, has made genetic editing more feasible and efficient. This method looks for a target sequence of DNA, using a complementary single-stranded guide RNA. The complex then creates a double stranded break at the target location and enables DNA repair off of an introduced donor DNA to replace the target sequence with a preferred mutation. This technique is scientifically exciting because of its versatility and facile targeting of a multitude of sequences. This advancement has shown that genetic editing could feasibly be utilized as a clinical treatment. This future possibility has sparked numerous discussion on whether or not we should be genetically editing in the first place.

Individuals base their opinions on a variety of sources, but one major source that informs opinions on this moral decision is religion. Genetic editing can only be helpful if it is acceptable and therefore applicable to possible users. PEW conducted a study on the potential usage of gene-editing amongst different categories of the American public (Street, NW, Washington, & Inquiries, 2013). An analysis of this data has shown that highly religious Americans are “much more likely than those who are less religious to say they would not want to use gene-editing technology in their families”(Funk, Kennedy, & Sciupac, 2016). This is important as it highlights the patterns and trends within public attitudes about gene-editing and confirms the notion that current genetic technologies are not accessible to religious communities.

Since religion can be a major contributor to a person's evaluation of genetic editing, if clinical benefits and care are to be extended, there is a need to develop techniques with which individuals can potentially agree. In addition, scholars have discussed the need for more conversation about genetics within faith communities as an attempt to answer any clarifying questions and produce a more transparent relationship between the two communities (Joseph, 2016). This project builds upon this plea for more well-informed conversations about genetics within religious communities, by going further and working on how the scientific community can also benefit by information about religious communities and not just the other way around.

This thesis will look specifically at the more conservative Christian discourse on genetic editing and possible paths for genetic editing research that would be more accepted by Christian communities. The general understanding of the Christian stance on genetic editing is that the Christian tradition simply does not approve it. However, this generalization fails to account for the complexity of Christian concerns and the opportunity for potential acceptance within the tradition. In order to deduce potential acceptable paths of genetic editing, I identify four "tension zones" (beginning of life, Creator-human relationship, imago *Dei*, and stewardship) within the Christian discourse. These tension zones are areas within the discourse that are both important concerns within the Christian community, but also house contradictions and potential avenues for acceptance of genetic editing. I argue within those four tension zones, the Christian stewardship concern of harm and the need for the conjugal act in the creation of new life are specific areas that

should be taken into consideration in order to find Christian-acceptable genetic editing research paths: epigenetics, somatic gene therapy, and *in vivo* genetic editing. In culmination, I propose an analysis of *in vivo* and *in vitro* CRISPR-Cas9 efficiency in regards to on- and off-target rates; on-target rates measure the amount of correct mutations of the intended gene sites, while off-target rates measure the amount of mutations of unintended gene sites. A reduction of off-target effects could potentially make a genetic editing method more clinically viable, as the potential "harm" or risk associated with the technique would be lower.

This is a dual religious studies and biology thesis and incorporates both fields of study to form a comprehensive analysis of Christian discourse of genetic editing and its relevancy to the scientific study. A greater goal of this thesis is to motivate the scientific field to consider the concerns of the potential users of their techniques and thus, produce more acceptable and comfortable clinical methods and therapies. This then has the potential to greatly increase the accessibility of clinical genetic editing, in terms of whether or not individuals will feel comfortable with their genetic editing techniques and consider it a viable option. I hope to show the benefits of this interdisciplinary approach and spark more discussions between the scientific and religious communities, that normally tend to be separate.

CHAPTER ONE.

CHRISTIAN STANCES ON GENETIC EDITING:

RESISTANCE AND OPENNESS

Introduction

Sitting in the waiting rooms of obstetrics and gynecology medical facilities are countless individuals who are not only thinking about Pap smears, STI testing, fertility, pregnancy and birth control, but today have to think about questions regarding genetic testing and editing. With the rapid development of genetic technologies such as CRISPR-Cas9 genome editing, the genetic editing of embryos is more realistic than ever before. These questions about genetic editing exist not only at OBGYN offices, but also in segments of life that pertain to one's own life instead of future children. Genetic technology research ranges from the genetic editing of eggs and sperm to discovering the intricacies of cancer through genetic events (Sánchez-Rivera et al., 2014, p. 428).

The common misunderstanding of the Christian stance on genetic engineering is that the tradition simply does not agree with it; however, this quick assumption ignores the distinct complexities within Christian dialogue surrounding genetics and the possibilities for developing more acceptable forms of genetic engineering. Although there are similar Christian concepts used in arguments for and against genetic editing, the way they are used can contradict each other. These contradictions present within the discourse highlight tension zones within the Christian tradition which contain particularly complex or contradictory understandings. These zones can be utilized by scholars when developing novel avenues of future genetic research, as there are multiple Christian interpretations to possibly align with.

I argue that the Christian discourse of genetic editing is rooted in four major concepts (the beginning of life, Creator-human relationship, *imago Dei,* and stewardship) and that although these concepts inform individual arguments, the defining factor between support and rejection is the perception of genetic editing in relation to harm; support of genetic editing is grounded in the categorization of genetic editing as a reduction of harm, while rejection is due to the categorization of it as inflicting harm. Two major concepts utilized by Christian arguments against genetic editing are the beginning of life and the Creator-human relationship. However amongst these, each argument has a uniquely nuanced understanding of the Christian concept. While still being informed by the Christian understanding of the beginning of life and Creator-human relationship, some arguments that support and reject genetic editing incorporate the concepts of *imago Dei* and stewardship. Although these two categories have arguments against genetic editing, here I also locate Christian support for genetic editing, which is a product of understanding genetic editing as reducing harm in some way rather than inflicting harm

Because of the notion that the Christianity and genetic editing occupy completely separate spaces, there is a significant lack of communication between religious and scientific communities. This has led to misinformed preconceptions regarding both the mechanisms and opinions of genetic editing. Thus, genetic editing research has evolved in heavy isolation from religious communities, producing genetic technologies that are scientifically intuitive but not as widely accepted by the religious public. Genetic editing research is only beneficial if it can actually help people, which can only happen if the techniques are accessible to a

broad patient spectrum. This chapter's work in identifying individual Christian arguments about genetic editing, locating the tension zones, and highlighting the defining harm factor between rejection and support of genetic editing, is important as it starts the journey towards producing genetic technologies that can possibly be more accepted by a religious public.

Thus in this chapter, I present individual Christian arguments for and against genetic editing. As mentioned, arguments about genetic editing make use of four major Christian tension zones: beginning of life, Creator-human relationship, *imago Dei*, and stewardship. The use of these concepts creates tension whereby the arguments utilize a general concept in contradictory or different ways. In what follows, the individual arguments are presented under the tension zone in which they fall. Their rejection or support of genetic editing is explained based on whether genetic editing is categorized as reducing or inflicting harm.

Beginning of Life

Within the medical field there is a very present debate on what stage life begins: *Is a zygote a bundle of cells or a unique life? Can a fetus or embryo be alive if it is not self-sustaining?* Although this is a debated topic amongst medical professions, the conservative Christian understanding of the beginning of life is rather concrete: at the moment of conception. In its ethics statement, the Christian Medical & Dental Association writes that "the Bible states that human life begins at the absolute beginning or inception using the term 'conception'"; and in order to clarify any confusion about the moment of conception, situates it as "the point of fertilization". (Christian Medical & Dental Associations, 2006, p. 14). This

understanding is also at the root of the Catholic Church's teachings on the care of human life, instructing a "protection of life in all its stages, from the first moment of conception until natural death" (Benagiano & Mori, 2007, p. 162). The Christian understanding of the beginning of life is specifically relevant to the topic of genetic editing, as it informs the majority of Christian arguments rejecting and supporting genetic editing. This section will analyze how two arguments utilize the Christian beginning of life as the primary reasoning for the rejection of genetic editing.

Within the Christian discourse of genetic editing, arguments against the use of embryo-derived stem cells within genetics research heavily draw upon the understanding that life begins at conception. Stem cells are a unique type of cell that can "reproduce themselves, and can also generate daughter cells that become differentiated cells" (Slack, 2012, p. 3). These differentiated cells then come to form specified units of cells that develop into different parts of the body. Stem cells are particularly useful within genetics as they provide a "self-renewing population of cells and thus may reduce or eliminate the need for repeated administrations of the gene therapy" (U.S. Department of Health & Human Services, 2001). If a gene is modified within a stem cell, the subsequent cells and body units that are derived from that initial cell will also carry that modification; this mechanism is the basis of the administrative and research benefit of stem cells in genetics. There are two major categories of stem cells: adult and embryonic. Although stem cells can be derived from both sources, there are two major benefits of using embryonic stem cells: they are easier to extract and culture; and they are able to differentiate into a more a variety of cell types. One major Christian argument against stem cell usage within

research is that it would entail the “death” of a fetus or embryo; this argument fails to incorporate adult-stem cells that are not embryo-derived. Rooted in the Christian notion of the beginning of life, the U.S. Conference of Catholic Bishops states that “every living member of the human species, including the human embryo, must be treated with the respect due to a human person” (U.S. Conference of Catholic Bishops, 2004). Under this model, any use of stem cells “wraps the user in a state of complicit guilt, because the only way the stem cells could be used is at the earlier expense of an embryo or fetus” (Modell, 2007, p. 173). This rejection of genetic editing techniques due to its usage of stem cells is grounded in the specific Christian understanding of the beginning of life being at the moment of conception, which then categorizes an embryo as living and embryo-derived stem cell techniques as “killing” lives.

While still utilizing reasoning rooted in the Christian beliefs about the beginning of life, some arguments against genetic technologies include the conjugal act as a necessary precursor for the creation of life. According to the Vatican, the conjugal act is established by God and serves both as unitive for the married couple and procreative (Pope Benedict XVI, 2006). In a letter addressing scientific advancements, the Vatican International Theological Commissions writes that “if a technique is used that does not assist the conjugal act in attaining its goal, but replaces it, and the conception is then effected through the intervention of a third party, then the child does not originate from the conjugal act which is the authentic expression of the mutual gift of the parents” (Vatican International Theological Commission, 2004). Here, the Vatican is addressing a specific scientific

advancement used within genetics, *in vitro* fertilization (IVF). IVF is clinically used as a reproductive technology that fertilizes a mature egg with sperm outside of the body and in a lab. This now fertilized egg is then implanted in the female's uterus in hopes of maturing into an embryo (Sher, Davis, & Stoess, 2005, p. 64). This technology is useful for genetic techniques as it can be used in order to easily modify the genes of the, sperm, egg, and fertilized egg. IVF is currently not compatible with Vatican's necessity of the conjugal act in the beginning of new life, as it circumvents any necessary physical sexual intercourse between the married couple. Thus, the Vatican frames one of its arguments against genetic editing in its usage of IVF and the creation of life without the God ordained conjugal act.

Within the Christian discussion of genetic editing, many religiously based arguments draw from the Christian understanding of the beginning of life at the moment of conception and induced by conjugal act; although these arguments are building upon the same general concept, they are calling into action different components of the beginning of life. This complexity in Christian reasoning allows for those within the tradition to align or disagree with any one of the many varieties within the discourse, while still overall holding a Christian stance on genetic editing. This complexity within elements of the Christian beginning of life still lead to a rejection of genetic editing, however. An understanding of these objections can be important for future genetics researchers to keep in mind as they research new genetic technologies and mechanisms.

Creator and Human Relationship

The perception of what being a human entails can vary from community to community. The Christian understanding of the elements of being a human is deeply attached to the understanding of God. Within the Christian tradition and specifically in the two creation story accounts of the Bible, God is defined as the Creator and the rest is God's creation. In these accounts, the God's creations are never said to independently create any other creatures. This characteristic Creator-human relationship has a stark divide between the two and allows little to none movement between the two roles. This firm relationship is relevant to this study of genetic editing debates, as it can serve as a basis to guide individuals' judgements of genetic editing based on whether it is compatible with the human's role or if it tries to "play" Creator. In order to understand how the Christian Creator-human relationship is utilized within the discourse, two major arguments will be analyzed within this subsection: Agneta Sutton's and Mathias Beck's rejections of genetic editing.

One major argument that utilizes the Christian understanding of the Creator-human relationship is Sutton's rejection of the use of genetic germline editing. Germline cells, such as eggs or sperm, are those cells whose genes are heritable and transmitted to an offspring. On the other hand, somatic cells are not heritable cells and can be found in the other various other parts of the body. Thus, germline genetic editing affects the future progeny. In her argument, Sutton addresses parents as her audience and urges them to reject germline genetic editing, as it both oversteps the Christian human role and fails to respect children as a gift from God. Sutton's understanding of the Christian hierarchical relationship is that humans do

not have the right or power to alter themselves and their lives; Sutton writes that "there are both physical and moral limits to our powers. As Christians we recognize that our lives are in God's hands and that ultimately we cannot save ourselves or others" (Sutton, 2012, p.153). Sutton expands on this understanding of God's power and the human lack of power when writing,

> Accepting children as gifts means not making their welcome depend on whether they satisfy standards set by us of health, ability, or beauty. At issue are human attitudes and aspirations that undermine the welcome of the child as a gift and deny it the respect it deserves as another person whose life comes to us as a gift from God. (Sutton, 2012, p.149)

For Sutton, children are gifts from God and creatures whose components are all God ordained and thus off-limits to human controlled modification. Any modification of them is considered a failure to unconditionally welcome God's gift and ultimately a form of harming this life. Using this understanding, any form of germline genetic editing is seen as a clear disrespecting and harming of God's creation, God's gift to humans. In this argument Sutton is specifically addressing parents and therefore only addresses germline genetic editing as it is extremely applicable to reproductive decisions. However, based on the understanding of the Creator-human roles used in this argument, God as the Creator and humans as gifts from God, it seems that Sutton would also oppose all genetic editing because any modification would be in fact modifying God's gift. Thus, Sutton utilizes an understanding of the distinct roles of God and humans and an understanding of modification as harm to support a

rejection of genetic editing should. For Sutton, genetic editing is both an attempt of humans overstepping the Creator-human divide and a mistreatment of God's gift.

Looking at the creator-human relationship from another angle, Matthias Beck also positions the God-human relationship at the forefront of the rejection of genetic editing. Beck works within an epigenetic framework. Epigenetics is a relatively new sub-field of genetics that explores how environmental conditions can affect and change "gene activity without changing the DNA sequence" and can lead to "modifications that can be transmitted to daughter cells"(Weinhold, 2006, p. A163). These environmental conditions are commonly referred to as drivers or agents. Beck's explains his understanding of epigenetics when he writes,

> Bodily relation-events reach down to genetic linkages. Genes are not merely present, in their damaged or healthy form, but they interact, and they can be switched on or off. Only switched-on genes are effective. The mechanisms for such switching are only partially known so far. But it seems that the brain, and thus a person's thinking and feeling, is involved in them. (Beck, 2007, p. 79)

Although there are scientific studies that link traumatic emotional events to epigenetic modifications, Beck's description misrepresents this modification as something that a person can easily turn off/on just by thinking. Current findings in epigenetics identify possible drivers as: "heavy metals, pesticides, diesel exhaust, tobacco smoke, polycyclic aromatic hydrocarbons, hormones, radioactivity, viruses, bacteria, and basic nutrients"; these drivers and their epigenetic effects have been linked to a variety of illness and health indicators such as "cancers of almost all types, cognitive dysfunction, and respiratory, cardiovascular, reproductive,

autoimmune, and neurobehavioral illnesses" (Weinhold, 2006, p. A160). Although this is the scientific explanation of epigenetics, Beck describes epigenetic mechanisms when stating,

> Many diseases have genetic backgrounds. Defective genes, however, do not necessarily lead to subsequent illness. Genes have to be switched on or off. Only activated genes trigger pathological change. The human brain and all of man's thinking and feeling are intimately connected with such activations. We may thus conclude that both inner life and religious outlook on life are relevant to the origin and development of diseases. (Beck, 2007, p. 67)

Beck's understanding of the Creator-human relationship fuels his claim that epigenetic drivers are in direct derivatives of an individual's relationship with God and that this relationship is what dictates whether God "switches" the genes on or off. Therefore, the human modification of genes would not be effective in actually reaching a desired outcome, because God has the "real" power over genetic activation. Under this model, genetic editing as a way of medical therapy for diseases is not targeting the true source and therefore not reducing any harm to life. Instead, Beck is arguing for individuals to spend their time investing in their relationships with God, as that is the initial cause; God has the power to then turn on or off the selected genes.

The Christian understanding of the Creator-human relationship utilized in both Sutton's and Beck's arguments describe a firm hierarchal divided relationship between humans and God; God has the control over the creation and the creation do not have control to modify themselves. Sutton adds a complexity to this

understanding by claiming that humans are not only creations but also gifts from God that are warranted an unconditional welcome into the world. This then positions genetic editing as both a breach of the Creator-human divide but also as a mistreatment and harm of the lives of God's creation. Beck includes an epigenetic element to the Creator-human relationship that explains epigenetic events as direct products of a stressed Creator-human relationship. Genetic editing is then understood as an ineffective approach to reducing any harm caused by genetic conditions; instead, Beck advocates for individuals to fix their relationships with God which will then allow for the possibility of God turning on or off particular genes. Even though both arguments each employ elements of the Creator-human relationship as evidence for the rejection of genetic editing, there is diversity in what sub-elements regarding the reduction or accretion of harm are utilized.

Imago Dei

In the Christian tradition not only do the concepts of the beginning of life and the Creator-human relationship guide individuals' judgements of genetic editing, but also the Christian concept of *imago Dei* ("image of God") plays a major role in the Christian rejection and support of genetic editing. A common biblical verse that informs the community of *imago Dei* is Genesis 1:27, "So God created man in His own image, in the image of God He created him; male and female He created them" (New International Version). *Imago Dei* informs the Christian understanding of the nature of human creation and identity as direct expressions of God. This concept manifests itself in Christian life as it expresses a "foundational relationship between God and man, with implications for properly appreciating basic human goods and

human flourishing, which in turn have repercussions for medical decision-making" (Cherry, 2017, p.219). Specifically, *imago Dei* is used within the Christian discourse of genetic editing in three major ways: *imago Dei* as a necessary understanding that prevents development of immoral reproductive ethos; *imago Dei* as the basis of the categorization of genetic editing as a mistreatment of a gift that is made in the image of God; *imago Dei* as the reasoning behind a support of genetic editing as a means to help individuals express their true identity.

When addressing a wide range of reproductive medicine that includes prenatal genetic testing, *in vitro* fertilization, and abortion, some arguments rely on *imago Dei* as a necessity that prevents individuals from developing a misinformed stance on reproductive issues. Associate Senior Editor of *Christian Bioethics,* Mark Cherry expresses the importance of *imago Dei,* when writing "Reference to the Imago Dei expresses a foundational relationship between God and man, with implications for properly appreciating basic human goods and human flourishing, which in turn have repercussions for appropriate medical decision-making" (Cherry, 2017, p.219). He later claims that when the Christian or general body forgets that all humans are made in the image of God, an ethical reproductive ethos forms that "normalizes not only contraception to control when to have children and medical selection of embryos for desirable traits through assisted reproductive technology, but also prenatal diagnosis and selective killing in utero of children with a likelihood of disabilities or undesirable genetic characteristics" (Cherry, 2017, p.224). This argument utilizes not only *imago Dei*, but also the Christian understanding of the beginning of life at conception. For Cherry, *imago Dei* is a necessary concept to

integrate into one's own moral judgement that leads to the development of morally sound medical decisions. Also, *imago Dei* manifests in Cherry's rejection of any categorization of desirable and undesirable traits. For Cherry, all elements of an individual, whether desirable or not, are representations of God. The understanding of the beginning of life informs Cherry's rejection of prenatal diagnosis that can lead to abortions and family planning methods that he understands to be selecting a promising embryo and "killing" the others. The blending of the two elements, beginning of life and *imago Dei,* continues within Cherry's rhetoric and specific usage of "children" instead of fetus or embryo. Cherry disapproves of individuals judging characteristics as desirable or undesirable, because each and every characteristic is made in the image of God. Here we can see Cherry's understanding of embryos and fetuses as already independent lives and souls and the removal of them as "killing". Cherry's disapproval of genetic editing is based both in the Christian understanding of the beginning of life and *imago Dei* and Cherry's understanding of genetic editing and possible endings of pregnancies as inflicting harm on these lives.

In a letter commenting on current scientific advancements, the Vatican International Theological Commission addresses genetic technologies and specifically the genetic editing of humans. Here the Vatican relies heavily on the concept of *imago Dei* as the essential nature of humans to inform a rejection of genetic editing based on a failure to value that human qualities are all made in the image of God. In this letter the Vatican positions *imago Dei* as the center piece for its framework and explains it as the key to a biblical understanding of "human nature

and [key] to all the affirmations of biblical anthropology in both the Old and New Testaments. For the Bible, the *imago Dei* constitutes almost a definition of man: the mystery of man cannot be grasped apart from the mystery of God" (Vatican International Theological Commission, 2004). For the Vatican, this defining characteristic of humans informs its opinion against the genetic editing of humans and is further explained when the Vatican writes, "A right to dispose of something extends only to objects with a merely instrumental value, but not to objects which are good in themselves, i.e., ends in themselves. The human person, being created in the image of God, is himself such a good" (Vatican International Theological Commission, 2004). *Imago Dei* is an element of being human that sets humans aside from other objects on earth. The Vatican's rejection of genetic editing is founded in the concept of *imago Dei* which informs the conclusion that human genetic modification would be an infliction of harm as it would be changing intrinsically good characteristics.

On the other hand, the notion of the full expression of an identity that is made in the image of God is utilized in support of genetic editing. Although contradictory to the Vatican's previous rejection of genetic editing, within the same letter the Vatican advocates for an acceptance of genetic editing as a method to help people fully express their identity which may be obstructed by congenital diseases. The Vatican's very clear supportive stance on the genetic editing of congenital diseases stands out against the many Christian campaigns against finding a Down Syndrome "cure" (Curtis, 2011; Knight, 2017; Peoples, 2017). In this letter the Vatican claims that congenital diseases such as Down Syndrome negatively affect the identity of a

person both physically and mentally. Thus, the Vatican advocates for genetically altering these conditions as the modification would "help the individual to give full expression to his real identity which is blocked by a defective gene" (Vatican International Theological Commission, 2004). This particular framework positions Down Syndrome and similar genetic conditions as barriers that constrict the ability to fully express a person's God given identity. In this scenario, genetic editing acts as a mechanism to remove the "defective gene" and therefore, reduce the harm caused by the disease preventing a God-given right to a full expression of identity. Although this is contradictory to other statements given by the Vatican, this support of the genetic editing of congenital diseases highlights the possibility of the Christian support of genetic editing when it is understood as a reduction of harm.

The concept of *imago Dei* is a major factor in many Christian arguments about genetic technologies, however the arguments each utilize different details of *imago Dei* to support or reject genetic editing. Cherry's argument centers in on the concept of humans being made in the image of God; in his view, forgetting this essential truth leads to the development of morally corrupt stances on reproduction. These stances would then lead to medical decisions that Cherry believes are harmful to current and future lives such as abortion and editing undesirable genetic characteristics. The Vatican's anti-genetic editing argument addresses genetic editing by specifying that changing an essential part of a human ceases to acknowledge the holy nature of humans being made in the image of God and would thus be inflicting harm on the individual. The Vatican's pro-genetic editing argument positions congenital diseases as barriers to a full expression of an *imago Dei* identity

and genetic editing as a mechanism to help reduce the harm cause by those diseases and allow full expression of identity. Although all three arguments incorporate the concept of *imago Dei,* their rejection or support of genetic editing is due to how *imago Dei* informs their judgement on what exactly is inflicting or reducing harm. For example, a condition may be considered to inflict harm on an individual if they understand this condition to be preventing the individual from fully living out their true identity. However, if the individual understands all characteristics as being made in the image of God, then any modification of that condition could be considered a disrespect or mistreatment of something truly *imago Dei.*

Stewardship

Within the Christian understanding of the hierarchal Creator-creation relationship, humans are entrusted with the responsibility to be stewards. This role and its application in today's scientifically advanced world has allowed for more variety of stances on genetic editing. Two common biblical passages that inform the Christian tradition on stewardship are Luke 12:41-48 and Matthew 25:14-30. The Luke parable informs on how to be a good steward through an analogy of a master's manager in charge of his servants and how to care for them. The passage from Matthew is the parable of the Bags of Gold in which a master gives his servants bags of gold in which the good and faithful servant invested his money so that when the master came back to collect the gold he gave more than was initially allotted to him. However, the "lazy servant" hid the money in a hole so that when the master came back he only gave back the same amount initially given to him. Both of these stories provide messages of taking care of God's creations and of cultivating God's

gifts so they are more abundant in the future. In a letter from the United States Conference of Bishops on how to be a Christian steward, stewardship is explained as "respect for human life—shielding life from threat and assault, doing everything that can be done to enhance this gift and make life flourish" (United States Conference of Catholic Bishops, 2006, p. 451). This understanding of stewardship within the greater Creator-human relationship is relevant to Christian genetic editing discourse, as it can influence individuals' judgements on genetic editing and whether or not it is proper care of God's creation. This section will analyze three different Christian arguments about genetic editing that utilize stewardship in their claims that genetic editing either harms individuals and is therefore not proper stewardship or helps protect and enhance individuals and is therefore appropriate stewardship.

The use of stem cells within genetic research and clinical applications of genetic technologies is a Christian moral dilemma that calls into question whether humans are fulfilling the role of stewardship. Public Health scholar Stephen Modell attempts to situate genetic editing within the Christian understanding of stewardship in regard to its usage of stem cells. Modell understands the acquisition of stem cells to be from either germline cells of aborted fetuses or fertilized eggs not used during *in vitro* fertilization and considers both methods as including the "death" of a fetus or embryo. The choice to use the word "death" when describing the termination of fertilized eggs exemplifies elements of the Christian understanding of the beginning of life fueling the argument; for Modell the fertilized egg, embryo and fetus are all alive. Thus he claims that "downstream use of stem cells wraps the user in a state of complicit guilt, because the only way the stem cells could be used is at the earlier

expense of an embryo or fetus" (Modell, 2007, p. 174). For Modell, any use of stem cells is intimately connected to the death and harm of lives. Since genetic technologies can rely on stem cells for their research, the clinical use of genetic editing cannot be separated from its harm to lives; thus, positioning it as counter to the stewardship role awarded to Christians.

Within the discussion of whether current genetic technologies fulfill or oppose the Christian call to stewardship, the care of not only the current and immediate next generation but all future progeny are included. Philosophy and Biomedical Ethics scholar James Delaney explains the Catholic Church's position on genetic engineering when writing that the Catholic Church distinguishes "between somatic cell therapy and germ line cell therapy, and prohibits the latter, because of two reasons: its potential to harm progeny and its use is in conjunction with *in vitro* fertilization" (Delaney, 2009, p.33). Although this may seem like a rejection of genetic technology as we currently know it, Delaney goes on to describe the very concrete changes within genetics that would allow for Catholic approval:

> Should the current state change in the following two respects, 1) risks to progeny are reduced so as to be out- weighed by likely therapeutic benefits, and 2) the subjects involved in the germ line therapy (either gametes or early stage embryos) do not affect persons coming into existence through a morally licit act (the conjugal act between a husband and wife), the Church's position on germ line therapy would likely be that it is morally permissible. (Delaney, 2009, p.33)

Delaney applies the call to stewardship to all current and future progeny by arguing that when the benefits of genetic editing outweigh the risks to currently non-existing lives, then germline genetic editing will be closer to being accepted by the Catholic Church. In addition, elements of the Christian understanding of the beginning of life complicate the Catholic Church's position on genetic editing. It seems that for Delaney embryos or fetuses not formed through conjugal act, such as through *in vitro* fertilization, are in fact not considered alive; this understanding is drawn from Delaney's second condition about persons coming into existence through a morally licit act. Therefore, the use of them can possibly be warranted because there is technically not harming of life. Throughout the article Delaney continuously critiques germline editing, which hints at Delaney not rejecting somatic gene editing as it would not entail the death of embryos or harm to future progeny. The current rejection of existing mainstream genetic technologies and the presentation of qualifications for potential acceptance of future genetic technologies both utilize the potential harm of progeny and harm of current lives as major factors for evaluation; thus, potential harm of progeny and embryos/fetuses should not be considered a "dead end" for genetic advancements but rather considered an avenue for potential acceptance.

Although stewardship has been used as criteria for the rejection of genetic editing and the proposal of changes to current methodologies, it can also be used as a framework to advocate for the usage of genetic editing. Rooted within a Christian pro-life framework, political scientist and biologist Brendan Foht focuses on the stewardship of fetuses and advocates for the usage of genetic editing as it can

increase the chances of survival for a fetus. Foht positions the analysis of genetic editing in contrast to abortions that are informed by prenatal screenings when writing, “Morally speaking, editing the genes of embryos rather than destroying them would be a step in the right direction” (Foht, 2016, p. 12). Foht considers genetic editing as a preferable alternative to the abortion of fetuses with unwanted characteristics. Here elements of the Christian concept of the beginning of life inform Foht’s understanding that embryos are in fact alive and the termination of them is “killing”. In conjunction, Foht’s analysis of the harm in each case facilitates his approval of genetic editing as it would in actuality reduce the harm of a life that would have been ended otherwise.

The Christian duty of stewardship is called upon throughout debates over the use of genetic technologies. Although God’s call for humans to be stewards is relatively agreed upon within communities, what exactly this stewardship calls for is not standardized. For Modell, stewardship, informed by the Christian understanding of the beginning of life, includes the protection of embryos and fetuses which allows for Modell to reject the use of genetic editing because it is a downstream use of stem cells and the “death” of embryos and fetuses. Delaney utilizes the concepts of the beginning of life with the conjugal act and stewardship when concluding the current germline genetic technologies both harm future progeny and utilizes “killed” embryos and fetuses; instead, Delaney proposes utilizing embryos not created via conjugal act and developing genetic technologies where the benefit will outweigh the risks of harm to future progeny. Foht also applies stewardship to fetuses but concludes that genetic editing would technically be a form of care and reduction of harm if it

increases the chances of survival. Intricacies within Christian stewardship such as caring for potential progeny, embryos, and fetuses allow for both Christian acceptance and rejection of genetic technologies; however similar to previously identified trends, the understanding of genetic editing as potentially protecting or reducing the harm of lives allows for a Christian justification of genetic editing.

Conclusion

There is a common misunderstanding that a unanimous Christian rejection of genetic editing exists; instead, there is a medley of rejections and acceptances with contradictions that highlight four major tension zones: the beginning of life, the Creator-human relationship, *imago Dei*, and stewardship. Aside from which of these different Christian concepts are utilized, the arguments within the Christian genetic editing discourse differ from each other depending on the understanding of genetic editing in regards to harm. All of the arguments that understood genetic editing to be inducing harm rejected genetic editing, while the arguments that supported genetic editing understood it to be a form of reducing harm. This judgement-call on whether or not genetic editing is causing or reducing harm is rooted in the individual's categorization of the genetic condition as being *imago Dei* or a barrier from fully expressing one's *imago Dei* potential. A combined understanding of what the tension zones are and the determining harm factor is extremely important for geneticists, as it can help them identify possible avenues to explore and then develop genetic technologies that may be accepted and used by more individuals. With this chapter locating the tension zones and harm factor amongst the current Christian discourse,

these tension zones will be analyzed for how they can specifically be useful in the development of new genetic technologies.

CHAPTER TWO.

FRUITFUL GENETIC EDITING RESEARCH PATHS

Introduction

A Christian married couple has been trying to have a child and participate in the Christian creation of life, however they were told by their physician that both of them are carriers of a congenital lethal disease. The couple is confused because they would still like to fulfill their marital calling to procreate but also fulfill their call to stewardship and care for their child's health, protect them from a lethal disease that could potentially kill them. One of the most common paths couples could chose is *in vitro* fertilization (IVF), which fertilizes a couple egg samples with sperm in petri dish and after enough division cycles, the healthiest or in this case the one without the disease gene is selected and the rest are discarded. However, this would not be a possible path for this couple as it would involve the ending of a life under a particular Christian definition (fertilized egg) and it would omit the conjugal act, also central to some Christian understandings of the production of life. Genetic editing is a potential path that the couple could consider as a way to possibly secure that their future progeny would not suffer from this condition. Genetic editing is the modification of an individual's genetic information (DNA) in efforts to modify a genetic characteristic or trait of theirs. Although genetic editing of humans is a relatively novel study, there have been recent major strides in the research of clinical application of genetic editing of human embryos. For example, in 2017 embryologist Shoukhrat Mitalipov successfully modified a human embryo that originally carried a heart defect gene (Ma et al., 2017). Mitalipov's team claims that this is a potential mechanism that would help "rescue mutated embryos that would otherwise be screened out of in vitro fertilization (IVF) procedures" (Servick, 2017). However, this specific method

would still not accomplish the Christian goal of ensuring the conjugal act in the creation of life. Within the Christian discourse of genetic editing, there is a concern regarding the necessity of the conjugal act as a mandatory precursor to the creation of life. As identified in the previous chapter, the Vatican claims that the conjugal act must not be replaced by but aided by reproductive technological techniques (Vatican International Theological Commission, 2004). The inclusion or reliance on the conjugal act within genetic editing research poses to hurdles. It does indeed complicate the experimental logistics when transitioning to human subjects, compared to simply mixing sperm and eggs in a petri dish. Secondly, scientists tend to look at efficiency and reliability when proposing new clinical methods; relying on the conjugal act is not seen as the most direct path. Thus, there exists a scientific need to fulfill a deficit within the research community that lacks major consideration of possible techniques that include the conjugal act. Research in these areas that would prove useful to and accepted by many individuals would include epigenetics, somatic cell genetic editing, and *in vivo* genetic editing research.

Epigenetics

As previously explained in the last chapter, the Christian concept of stewardship, which calls for individuals to take care of and nurture God's creation, fuels a call to protect current and future progeny from harm. Attending to the Christian concern about harm to progeny and the Christian calling of stewardship, Epigenetics is a specific field of genetics that focuses on hereditary gene functions that are not rooted in DNA sequence alterations; in other words, it focuses on structural changes that may affect the cell's access to DNA via physical markers

such as the extent of tight coiling of the genetic material. In a 2008 epigenetics conference, an epigenetic trait was defined as “a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence” (Berger, Kouzarides, Shiekhattar, & Shilatifard, 2009, p. 781). These epigenetic states of the genome can affect the accessibility of the genome and its expression. The three major epigenetic modifications are DNA methylation, histone modification and non-coding RNA- associated gene silencing. It is commonly understood that possible sources of epigenetic modifications can include one’s environment and diet. With epigenetics as a “buzz” field surfacing on the pages of multiple health blogs to scholarly scientific conferences, epigenetics has been incorporated into a diverse public discourse; it complicates the general understanding of hereditable information as predetermined and not affected by one’s own life. Although not the explanation for many genetic conditions, the epigenetic explanation of many gene expression conditions produces a sense of controllability regarding genetic conditions. Not only can lifestyle factors negatively affect gene expression, but certain lifestyle changes can instead benefit genetic expression and serve as epigenetic therapy that is not affecting the DNA itself. This ability to possibly “fix” one’s condition without having to physically change the DNA can facilitate individuals to feel more autonomous and capable of independently helping their condition and possibly no longer passing on a negative epigenetic marker onto their progeny.

To investigate common lifestyle variables and their possible epigenetic effects, studies have investigated both environmental and dietary variables. Regarding the environment, pollution is a major concern as an epigenetic

modification initiator of histone acetylation. Dinga R et. al investigated this relationship between pollution and histone acetylation in mice model and claims that it can be used to help further elucidate the relationship between air pollutants and lung disorders (Ding et al., 2016, p. 170). Pertaining to diet, a recent study concluded that a ketogenic diet can help alleviate the a deficiency of chromatin opening common in Kabuki syndrome (Benjamin et al., 2017). Here, diet is used as a form of epigenetic therapy to help reduce the existing epigenetic condition of the genome in mice with Kabuki syndrome. A research group focusing on acute myeloid leukemia (AML), has identified epigenetic mutations that result in a pre-leukemic cell state (Wouters & Delwel, 2016). These researchers then identified that epigenetic therapy to this pre-cancerous state could help reduce the cases of this disease. These studies are all pre-clinical and producing vital information for future clinical epigenetic therapy. Current human epigenetic clinical trials include the development of histone deacetylase (HDAC) inhibitors for cancer therapy and neurological disorder treatment (Marks & Xu, 2009)(Kazantsev & Thompson, 2008). These inhibitors affect the chromatin packaging and expression of genes in charge of cell cycle events that are usually abnormally regulated in cancer cells (J. M. Wagner, Hackanson, Lübbert, & Jung, 2010).

Although these clinical and pre-clinical trials show very promising results for the efficiency of epigenetic therapy, the easy reversibility of epigenetic changes lends itself to both the risk and benefit of the clinical use epigenetics as therapy. If an unintended epigenetic modification does occur during a treatment, it is very possible that it could be easily reversed. However, the opposite case of an

epigenetic beneficial modification being reversed could be considered a risk. *Is the efficiency of epigenetic therapies reduced because of this reversibility and how long would therapy effects last?*

In regards to the specific Christian understanding and moral decision about epigenetics, very few have published any specific opinions on it. As noted in chapter 1, one published Christian understanding of epigenetics understands the epigenetics markers as heritable effects of sin that can act as pre-dispositions to sin for future individuals (Beck, 2007). Due to the overall lack of published Christian arguments about epigenetics, I will focus on how epigenetic research and therapy might interact with the existing Christian themes and concerns of stewardship and prevention of harm.

With its ability to help treat potential hereditary health complications and since epigenetic modifications are technically not changing the DNA material itself, epigenetic therapy appeals to the Christian theme of stewardship and concern about harm to progeny. Epigenetic therapy would allow individuals to remove/reverse harmful epigenetic markers from their genetic material without actually changing the DNA and thus without overstepping the Creator-human boundary. Such treatment would allow Christian individuals to "take care" of their future progeny and thus be stewards of God's gifts. In addition to helping fulfill the Christian concern regarding the care and prevention of harm to future progeny, epigenetic therapy would address the Christian understanding of genetic conditions, discussed in the previous chapter, as linked to environmental stressors that are in fact resultant from the individual's relationship with God (Beck, 2007, p. 67). In this case epigenetic therapy would be

addressing a physical component of a person's hereditary material, but would still appeal to Christian epigenetic and environmental understanding of certain conditions rather than purely DNA based.

Although there is an abundant amount of epigenetic research already being conducted and in the pipeline, an interesting epigenetic variable that would be important to understand more comprehensively through more research is the longevity of certain epigenetic mutations. This understanding of how long epigenetic modifications last before being reversed or even turned into something else can be important in deciding whether or not epigenetic therapy is clinically worthwhile.

Somatic gene editing

Another potentially productive genetic editing avenue to research is somatic gene editing, which appeals to the Christian stewardship call to prevent harm to progeny. As discussed in the previous chapter, somatic cells differ from germline cells in regards to heritability; somatic cells are not heritable and comprise the far majority of an organism. In theory, the modification of a somatic cell could affect a variety of somatic cells targeted but would not be transmitted to future offspring, as it would not be modifying any germline cells. In this section I will look at both current practices of fetal and adult somatic cell editing, the risks involved, and how they can alleviate some Christian concerns.

Fetal somatic cell editing is an *in utero* method and has been identified as a potential "prenatal prevention and permanent correction of disease manifestation particularly for early onset diseases" (Coutelle & Rodeck, 2002, p.670). Coutelle and Rodeck both identify *in utero* fetal somatic gene therapy as not only an excellent

preventative medicine option, but also an excellent option for individuals who find themselves having to consider pregnancy termination following the diagnosis of a severe genetic disease. The option of *in utero* fetal somatic gene therapy would allow them to instead edit this unwanted and harmful genetic disease, while still allowing them to keep the same fetus alive and not have as much of a pressure to consider abortion. Douar et al. investigated and solidified the role fetal somatic gene therapy can have in the prevention of irreversible perinatal diseases (Douar, Themis, & Coutelle, 1996, p. 633).

A more recent study investigated easier ways to treat congenital diaphragmatic hernia, a condition that ultimately can hinder proper lung formation. Currently this disease is treated via a physically invasive endoscopy of the fetal trachea, which can be risky when done on delicate fetal membranes. Using the sheep as their model organism, the researchers concluded that sheep fetal trachea can be accessed and genetic therapy can be administered to it by vectors via percutaneous ultrasound-guided injections (David et al., 2003, p. 385). Basically, the researchers are proposing that an improved method to the fetal endoscopy would be to inject genetic editing material into the fetal trachea via a tiny needle and that the genetic editing material will be guided to the target area via specific wavelengths applied to the subject. This finding is exciting as it points to a near future where individuals will be able to "better protect" their progeny through genetic editing methods, as they could potentially be less prone to physical complications such as membrane rupture. In addition to research of fetal somatic gene editing, there has been lots of research in adult somatic gene editing.

Current research and clinical interest in adult somatic gene editing ranges from understanding the nature of cancer to metabolic diseases. In order to more comprehensively understand cancer, Sánchez-Rivera et al. performed a rapid modelling of cooperating genetic events in cancer through somatic gene editing (Sánchez-Rivera et al., 2014, p. 428). This modelling allowed them to better identify which mutations could be causing the tumorigenesis within a mouse model. In this study, the research team looked at common identified cancer-related genes within a lung-cancer model and systematically activated or deactivated them with genetic editing and observed its effect on tumorigenesis. The hope of this study is that a dense catalogue of cancer genome mutations can help identify early stages within the cancer timeline and subsequently be used to develop therapies for individuals (mostly non-fetus) with cancer. Since a majority of cancers develop later in life, the applications of this therapy would have more opportunities within adults rather than only simply in fetuses. In regards to somatic gene editing's role in understanding metabolic diseases, it allows for an easier method of modeling. Currently researchers model metabolic diseases by genetically editing germline cells and breeding said cells to produce metabolically diseased model organisms. However, Jarrett et al. explored the possibility of using somatic editing to simply edit already existing adult individuals so that they express the desired mutation (Jarrett et al., 2017, p. 1). This method allows for an easier research method that opens up paths towards more feasibly research of metabolic diseases and therapies.

Somatic gene editing has clearly allowed for groundbreaking and helpful understandings of medical conditions and the nature of cells. The clinical use of

somatic gene editing allows for specific non-hereditary genetic editing that lacks the same risk to progeny that accompanies germline editing. However, this usage does come with some risks that are important to identify. First, although somatic gene editing in theory should not be transmittable to offspring, there is still an underlying risk of germline transmission. A research team from the Department of Obstetrics and Gynecology or Bern University Hospital, explains this risk as "Because vector integration into germ cells, if it occurs, is likely to be random, an integration event could potentially have disastrous effects for progeny conceived from such a germ cell" (A. M. Wagner, Schoeberlein, & Surbek, 2009). This same research team identifies another potential risk, maternal spread: "*the possible risk of vector spread from the fetus to the maternal body, although the number of layers may not be the only factor determining placental permeability"* (A. M. Wagner et al., 2009). Although current studies suggest this risk is relatively low, I agree with this research team in the necessity for more research in order to fully understand maternal cell transduction (Ye, Gao, Pabin, Raper, & Wilson, 1998). In my opinion, this potential risk to the mother seems like it would not sit well with the general and Christian population. However, when analyzing the Christian discourse of genetic editing, there are no major arguments pro or against genetic editing addressing risk to the mother. This then points to a seeming lack of attention towards women's health in regards to genetic editing within the Christian discourse.

In regards to the relationship between further development of somatic genetic editing and Christian discourse of genetic editing, somatic genetic editing could potentially help fulfill the Christian concerns regarding the safety of future progeny

and the need for the conjugal act in the creation of the new life. As highlighted in the previous chapter, the Catholic Church distinguishes “between somatic cell therapy and germ line cell therapy, and prohibits the latter, because of two reasons: its potential to harm progeny and its use is in conjunction with in vitro fertilization” (Delaney, 2009, p.33). In conjunction with the general understanding of a low germline transmission (Ye et al., 1998), somatic genetic editing allows for a safer method of treating genetic conditions without unforeseen future effects on offspring that are potentially possible in germline editing. With further research, adult somatic gene editing techniques could be developed that solidified the editing of cancerous tumors in adults via cancer cell targeted genetic editing materials, whether through ultrasound or other guiding mechanisms. In addition to appealing towards the Christian stewardship element of prevention of harm, somatic gene editing can appeal towards the Christian necessity of the conjugal act when procreating. The option of editing the somatic cells of a fetus, allows a Christian individual to both create life via the conjugal act but also, if the individual had been considering the pregnancy termination, this would allow another option that would be more accepted by the Christian public.

In addition to appealing to Christian concerns, somatic genetic editing appeals to bioethical and general medicinal goals. First, the ability to genetically edit the somatic cells of individuals, and specifically adults, situates itself well within bioethical concerns about agency. With adult editing, there is clearly more agency provided towards the individuals, in contrast to genetically editing the somatic cells of fetuses. The somatic cell editing of fetuses, on the other hand, seems to address

the concerns of the medical goal of pushing towards a future of preventative medicine. In 2002, 8 major healthcare member organizations, ranging from doctors to pharmacists, collaborated to implement healthcare objectives titled the “Clinical Prevention and Population Health Curriculum Framework” as a major effort to help the United States healthcare system shift from one of diagnostics/curative to one of preventative(RK Riegelman & Garr, n.d.). Somatic cell editing appeals to this model of preventative medicine, as it allows for the treatment of potential at risk cells before a major disease develops.

In regard to Christian, general medical, and bioethical concerns, further somatic genetic editing would prove productive in the development of more acceptable and therefore, usable forms of genetic editing. Further research into the amount of germline transmission present within somatic editing treatments would help elucidate the amount of potential harm to progeny there actually is; this can then increase the amount of information individuals have when deducing their potential medical paths when faced with genetic conditions. In addition, there must be further research into the potential maternal risk during fetal editing, both to increase the agency of the women undergoing this procedure but also to help further build a future within medical and Christian discourse that commonly incorporates women’s health and concerns.

In vivo genetic editing

Not only is the type of genetic information being edited important, but also the mechanism that it is accessed and edited is just as pertinent in regards to Christian acceptance. When it comes to the editing of progeny, whether before or after

conception, the method of fertilization utilized is important as there is a Christian need for to maintain a tradition understanding of conception through the marital conjugal act. The Christian pushback against some clinical fertilization techniques such as *in vitro* fertilization (IVF) are rooted in this exact concern that rejects the fertilization of the egg in a laboratory petri dish and advocates for the couple to instead procreate via heterosexual intercourse. This is relevant to this discussion of genetic editing as scientific and specifically genetic studies are commonly one of three types: *in vivo, in vitro,* and *in silico. In vivo* experiments are carried out within a living organism and is capable of producing a greater understanding of the total effects of a variable on the entire organism, including its total interaction with other body variables. *In vitro* experiments are the opposite of *in vivo* and are not conducted within a living organism. These studies produce a less comprehensive understanding of the particular independent variable's effect as it does not incorporate organismal conditions as well as *in vivo* studies. However, *in vitro* studies benefit from not having as much of a risk of harming actual organisms and are more easily reproducible. *In vitro* techniques are common amongst reproductive scientist in regards to egg/sperm extraction and *in vitro* fertilization, because it can provide a sterile, easily observable and controllable environment. *In silico* refers to studies performed using a computer software simulation and an abundant database. This is a relatively newer form of experiment that is commonly used as a primary investigation of a variable or mechanism and can help construct possible *in vivo* or *in vitro* studies of the same variable. In genetics, *in silico* studies are now ever present in gene expression analysis(Murray, Doran, MacMathuna, & Moss, 2007). With this

background of current experimental approaches, this section focusses on *in vivo* genetic editing of eggs (fertilized and non-fertilized) and sperm and analyzes it as a potential productive avenue of clinical genetic editing that will be more accepted by Christian users by appealing to the inclusion of the conjugal act.

Although not currently clinically used, *in vivo* genetic editing locates itself in a variety of contemporary research studies. *What does this current in vivo genetic research look like?* In 2015, Lukas Dow et al. published a study that examined inducible *in vivo* genome editing with CRISPR-Cas9 in mice and claims that an "inducible CRISPR (iCRISPR) system can be used effectively to create biallelic mutation in multiple target loci and, thus, provides a flexible and fast platform to study loss-of-function phenotypes *in vivo*"(Dow et al., 2015, p. 390). Dow's team essentially provides a viable approach to rapid and scalable studying of gene functions *in vivo*. Also using mice as their model organism, in 2011 Hojun Li et al. study how *in vivo* genome editing can restore hemostasis, the stopping of bleeding, in hemophilia, a genetic disorder that reduces an organism's ability to form blood clots and terminate bleeding. Before this study, the use of zinc finger nucleases (ZFNs) was used in genome editing *in vitro*, however this research wanted to explore its efficiency *in vivo*. By examining the effective level of gene targeting and concluding that it could effectively correct the hemophilic prolonged blood clot times, they were able to claim that this "ZFN-driven gene correction can be achieved *in vivo,* raising the possibility of genome editing as a viable strategy for the treatment of genetic disease"(Li et al., 2011, p. 217). This research team's finding show that ZFN can not only be used in vivo, but that it can efficiently bind the new desired gene to

the targeted location with the necessary consistency and efficiency to be a clinically viable method. With studies like the ones presented showing the viability and efficiency of *in vivo* genetic editing and the beneficial clinical applications, its future clinical use on humans is near and the risks of the clinical human application are important to consider.

With *in vivo* genetic editing, there is an inherent risk that accompanies the therapy being done within the living organism. The off target of gene editing is a measurement of unwanted genetic manipulations of the genome. This means that possible unwanted mutations could include the wrong gene being edited or the wrong cell/tissue type being edited. *In vivo,* if there is a mistake and a different gene is modified or if the wrong cell type is edited, it can possibly affect multiple unintended regions of the body. For example, if one wanted to target a specific gene and there was another gene that had a similar coding region to the target, the editing mechanism has a risk of binding to the unintended region. This would then result in an unintended effect, commonly called the off-target effect. If an individual wanted to target a controlled section of the body such as the eggs but the mechanism used traveled to another region and edited that, there could potentially be unintended effects. In contrast, *in vitro* editing mistakes could not travel to and affect other regions and these mistakes could be easily identified and those affected cells could simply not be used.

Although *in vivo* genetic editing research is very much present and abundant today, a majority of the research focuses on somatic cell editing, such as the cancer and metabolic disease studies discussed earlier. I propose that shifting some

attention to *in vivo* germline genetic editing would prove fruitful in efforts to produce more accepted genetic editing techniques. The next section will focus on *in vivo* germline genetic editing and investigate its efficiency compared to *in vitro* genetic editing. *In vivo* germline editing could manifest itself as editing the sperm or eggs themselves, or editing the cells that produce sperm and eggs. *In vivo* genetic editing of germline cells would potentially address the Christian concern for the conjugal act in the creation of future progeny, as it would allow for the editing of egg and sperm/sperm producing cells within an individual prior to the conjugal act. These techniques could be helpful for individuals who are concerned about a need for the conjugal act but want to fulfill their role as stewards and protect their progeny via protecting them from a certain heritable disease.

Conclusion

With the previously identified tensions zones within Christian discourse of genetic editing in mind, possible paths of genetic research can be identified to help develop more accepted genetic editing practices (epigenetics, somatic gene therapy, *in-vivo* genetic editing and adult genetic editing) that appeal to the Christian stewardship goal of prevention of harm and the need for the conjugal act in the creation of new life. Amongst the analysis of possible research paths, I identified a concerning lack of attention towards somatic genetic editing's potential risk of maternal transduction, when the editing mechanism vector spreads from fetus to mother. Although this lack of concern allows for more potential genetic editing paths that the Christian body could potentially accept, it should not go unquestioned. Further inquiries as to why exactly the Christian community is not worrying about the

maternal body when considering risk assessment should be considered. The next section will examine current data on off-target effects of CRISPR-Cas9 genetic editing and propose an experiment to understand if the off-target affects will change when *in vivo* verses *in vitro.*

CHAPTER 3.

AN ANALYSIS OF *IN VIVO AND IN VITRO* CRISPR-CAS9 EFFICIENCY

Introduction

Today, the pages of online health blogs to Time Magazine are filled with the general public in discussion of genetic editing. The focus of these conversations varies between bioethical concerns, efficiency, and safety for clinical application on humans. As discussed in the previous chapters, since the launch of CRISPR, genetic editing has been a hot topic within Christian communities; a major Christian concern with genetic editing and its usage is the lack of the "conjugal act" in current genetic editing of future progeny. For example, commonly paired with genetic editing of embryos is *in vitro* fertilization (IVF), which forgoes an explicit need for the heterosexual sex, but instead is efficiently accomplished with the fertilization of an egg with a sperm sample in a laboratory setting. In efforts to develop more forms of genetic editing that communities are comfortable with and therefore allow genetic editing to be more accessible to these patients, I propose that comparing the efficiency of *in vivo* and *in vitro* CRISPR genetic editing will help form a basis for further research and possibly development of *in vivo* germline editing. This form of genetic editing is the topic of analysis for this study, as it relieves a Christian concern about the need for the "conjugal act" in the creation of new life.

In order to attest to the efficiency of *in vivo* CRISPR genetic editing in comparison to *in vitro*, this study will analyze current research on *in vitro* genetic editing and suggested optimizations and then apply this information in the development of the experiential design. This proposal will offer a study that will analyze both on-target and off-target mutations, in addition to overall efficiency, in both *in vivo* and *in vitro* treatments.

On-target mutation efficiency

In order to test whether or not a genetic editing technique is in fact editing the intended sequences, the on-target mutation efficiency must be measured. Currently there are a variety of methods to do this: mismatch cleavage assay, high-resolution melting analysis (HRMA), heteroduplex mobility assay by PAGE, cleaved amplified polymorphic sequences (CAPS) analysis, Sanger sequencing, amplified fragment length polymorphisms (AFLP), and Fluorescent PCR-capillary gel electrophoresis (Zischewski, Fischer, & Bortesi, 2017). Each method has its own benefits and disadvantages but generally concern whether or not it can detect small indels, large indels, and substitutions. The methods that miss large indels are: HRMA and heteroduplex mobility assay by PAGE, while AFLP fails to detect small indels and Fluorescent PCR-capillary gel electrophoresis fails to detect substitutions. Although many kinds of mutations could be incorporated by HR depending on the donor DNA, small indels from NHEJ repair and substitutions from HR using a donor template are more common than large indels. This characteristic is because the CRISPR system relies on endogenous double-strand break repair pathways. Thus, HRMA and/or a targeted sequencing method could detect the mutations.

HRMA is an analysis method that concentrates on a target region, amplifies it with a fluorescent PCR and analyzes it via melting curves (Zischewski et al., 2017). This technique is dependent upon the fluorescence loss when the dyes are no longer attached to the dsDNA during thermal denaturation. These melting temperature curves are utilized to identify the specific nature of the allele (homozygous/heterozygous mutant/wildtype); the shifts of the curves represent a

variation of nucleotides (Thomas, Percival, Yoder, & Parant, 2014). In order to produce an optimal HRMA with high resolution, the amplicon size is suggested to be around 100 bp (Thomas et al., 2014, Zischewski et al., 2017). The same amplification products could later be used for other analysis methods because HRMA does not alter the sequence of the amplicons. If for some reason a large indel is expected, to account for the limitation of HRMA not detecting large indels, AFLP can be used to detect these larger mutations. The

Some decreases in on-target efficiency can also be attributed to nonhomologous end joining (NHEJ) of sequences rather than the preferred method of donor DNA integration through homology-directed repair (HDR). In order to combat this efficiency deficit, I will utilize single-stranded donor DNA (ssDNA) instead of the common double-stranded DNA (dsDNA) as ssDNA has been shown to increase HDR efficiency in Cas9-mediated gene editing in human cells (Richardson, Ray, DeWitt, Curie, & Corn, 2016). The donor ssDNA is developed to be of optimal length that is complementary to the 3' terminus of the cleaved DNA strand that is complementary to the target strand. This optimal donor ssDNA has been shown to increase HDR rates to up to 60% and should be incorporated within protocols when developing and/or investigating efficient methods of CRISPR genetic editing.

Off-target effects

Ways to detect

The rate of off-target mutations is an essential factor dictating the potential usage of a certain method of genetic editing. For the purpose of measuring the off-

target effects, there are both biased and unbiased methods of varying sensitivity. Biased approaches look at specific predicted sites, while unbiased approaches look at the whole genome. For this study, I propose to use a targeted sequence amplification, a popular biased method that amplifies and sequences previously identified segments that may contain off-target sites. These potential candidates for off-target mutations can be identified via sequence similarity to the Cas9 guide RNA sequence. Sequence analysis of amplified candidate regions can be performed via Sanger sequencing or next generation sequencing (NGS). Sanger sequencing becomes a bit impractical when there are many potential segments to sequence. Although the amount of off-target sites vary widely based off of the identity of the gRNA, the number of off-target mutations can range from 0-150 (Zischewski et al., 2017). Digenome sequencing is an unbiased technique which utilizes Cas9 and sgRNA *in vitro* to scan for potential off-target sites that can be used in the targeted sequence amplification, later sequenced via NGS (Zischewski et al., 2017). This method uses the combination of cell-free genomic DNA, Cas9, and sgRNA to identify both the target and off-target sites that are cleaved and measures the frequencies of unintended indels. Digenome sequencing has many benefits that include identifying off-target sites whose mutations occur at rate below 0.1% (Kim et al., 2015), incorporating multiple gRNA at once, and filtering out the cell's own introduced double stranded breaks (DSBs). In order to be computationally identified, the DNA is digested to produce sequence reads with the same 5' ends at cleavage sites (Kim et al., 2015). One identified setback of this method is that it is indeed being conducted *in vitro* rather than *in vivo* which could possibly lead to skewed

results (B. X. H. Fu, St. Onge, Fire, & Smith, 2016; Zischewski et al., 2017), but there is no evidence suggesting that off target effects for a particular guide RNA would differ between *in vitro* and *in vivo* systems.

Ways to minimize

Unlike Zinc Finger Nucleases (ZFN) and transcription activator-like effector nucleases (TALENs) which are dimeric, CRISPR-Cas9 is monomeric which leads to a higher likelihood of off-target mutations because it scans for shorter target sequences. In addition, the sgRNA used can lead to off-target mutations, as certain sgRNAs can tolerate different amounts of mismatches (Zischewski et al., 2017). Because sgRNA sequence and length dictates potential off-target sites, it is an optimal component to modify in order to reduce off-target mutations (Frock et al., 2015). For example, one could potentially minimize off-target effects by reducing the gRNA length from 20 nt to 17 or 18 nt to reduce the RNA-DNA binding energy (Y. Fu et al., 2013). Finally, the nuclease can be engineered to make a double-stranded break via 2 separate single-stranded cuts by targeting two nearby sequences with a Cas9 cleavage mutant (a D10A mutant nickase version of Cas9n) (Ran et al., 2013).

Methods and Experimental Design

Goal of study.

The goal of the study is to quantify differences in CRISPR genetic editing efficiency, in regards to on-target mutations, off-target mutations, and overall efficiency between *in vitro* and *in vivo* editing of mouse eggs.

Mouse model.

In order to examine the efficiency of CRISPR genetic editing of eggs, the utilization of a fluorescent mouse model allows for simple detection of efficiency by physical examination of cultured cells. The mice used in this experiment will have been bred to express green fluorescent protein (GFP) throughout their bodies through methods established by Ikawa et al. For the *in vitro* treatment, pre-existing mouse cell lines expressing GFP will be used (Ikawa et al., 1995). These GFP expressing mice will then be mutated into BFP expressing mice via CRISPR-Cas9 using HDR. Successful mutation will result in a blue fluorescence, while NHEJ will result in a loss of fluorescence due to the creation of small indels.

Guide RNA.

As indicated by Liang et al. (2017), the guide RNA (sgRNA) to target the GFP gene will be designed and synthesized using GeneArt™ CRISPR gRNA Design Tool and Synthesis Kit. Qubit® RNA BR Assay Kit will be utilized to compute the concentration of sgRNA needed.

Donor DNA.

In order to easily identify the efficiency of gene editing in this model, a blue fluorescent protein (BFP) gene will be used as the donor DNA. Taking into consideration previous research on the higher rates of HDR when using ssDNA in comparison to dsDNA as donor DNA (Richardson et al., 2016), this designed BFP donor DNA will be single stranded. A correct targeting of the GFP sequence and

integration of the BFP donor DNA will result in the mouse egg cells emitting blue light when excited.

CRISPR-Cas9 treatment.

In order to standardize the variables between the two treatments, the same CRISPR treatment system will be used for both. We will use the AAV serotype 8 (AAV8) as a vector for the CRISPR- *Staphylococcus aureus* Cas9 (SaCas9) complex with the GFP sgRNA and an oocyte specific promoter, Gdf9 (Salvador, Silva, Kostetskii, Radice, & Strauss, 2008) (Figure 1). The AAV serotype is an Adeno-associated virus that infects humans. This delivery system has been shown effective for the delivery of CRISPR-cas9 systems into mouse skeletal and cardiac muscle, with this particular smaller Cas9 variant (Nelson et al., 2016). The AAV vector complex will be either directly microinjected into the oocyte region of the mouse for the *in vivo* treatment or transduced to the collection of harvested egg cells for the *in vitro* treatment. For the *in vivo* treatment, identification and surgical exposure of the female mouse reproductive tract protocol will be used from a study exploring mouse ovarian fat pads (Flesken-Nikitin, Harlan, & Nikitin, 2016).

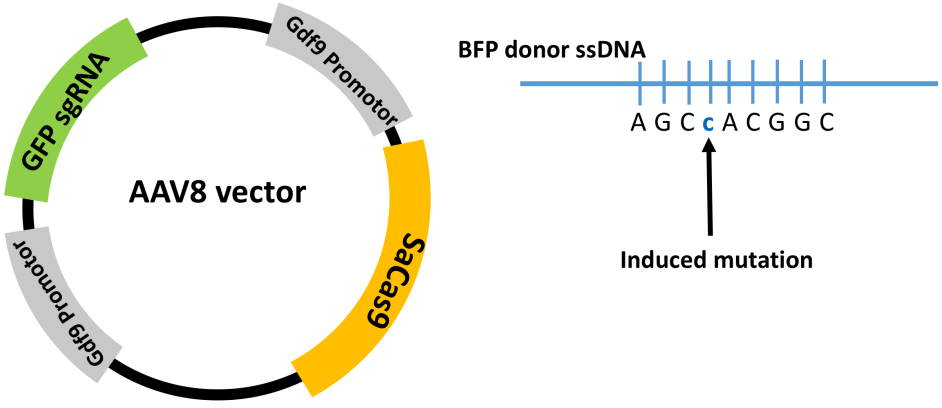

Figure 1. AAV8 vector and donor BFP ssDNA with induced mutation.

Off-target effects measurements.

A targeted sequencing approach can be utilized to measure off-target mutations in both *in vivo* and *in vitro* treatments. This approach will apply digenome sequencing to identify potential off-target sites that resemble the GFP sequence. Then, through targeted sequence amplification of the oocyte DNA, these targeted sites will be sequenced to determine if they were modified by the nuclease enzyme treatment. The oocytes used for amplification and analysis will either be from the *in vitro* CRISPR-Cas9 treatment or harvested from the *in vivo* treatment. The rates at which these potential off-target sites are modified in both *in vivo* and *in vitro* treatments can then be determined and compared (Figure 2).

On-target mutation measurements.

To determine efficiency of the two treatment methods in regards to on target mutations, FACS will be used to determine the rate of on-target mutations. For the *in vitro* treatment, the edited unfertilized oocytes will be analyzed through FACS for fluorescence expression. The *in vivo* edited oocytes will be harvested after CRISPR treatment and similarly analyzed via FACS for fluorescence under the appropriate wavelengths (Figure 2).

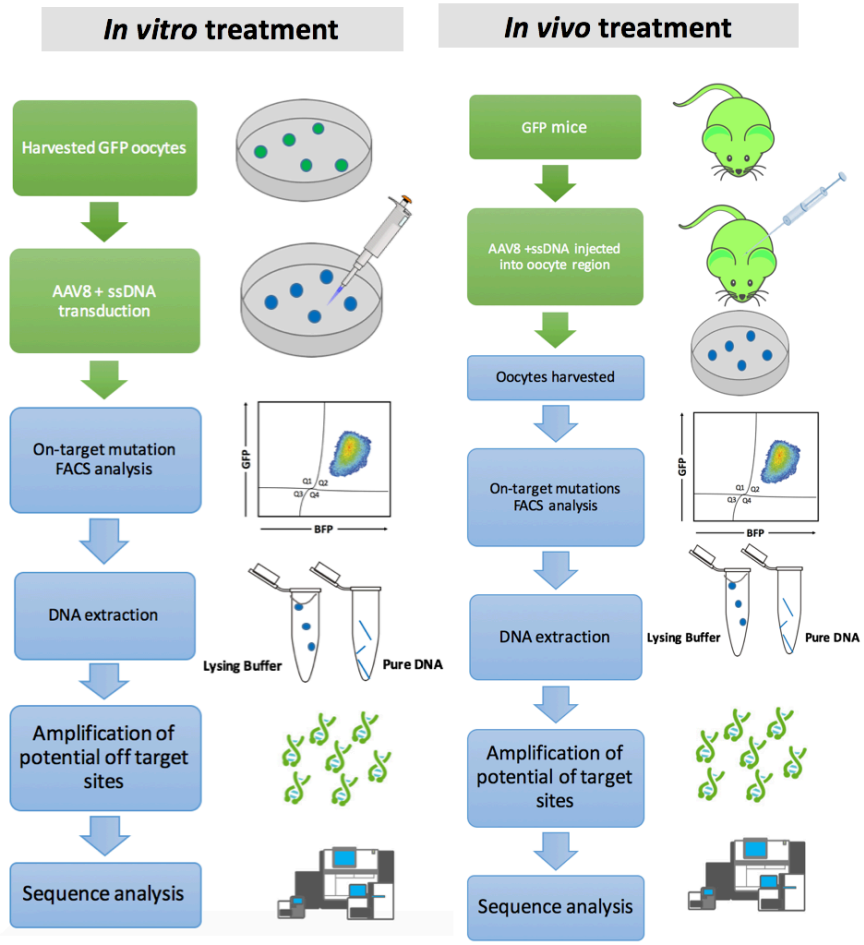

Figure 2. Experimental design flow chart for both *in vivo* and *in vitro* treatments.

Expected Results and Analysis

Off-target mutations.

Off-target mutation rates allow for a quantification of the likelihood of the CRISPR complexes to create an unintended mutation at a sequence other than the target. These rates will be obtained through targeted sequence amplification of the regions identified by digenome sequencing. A two-tailed t-test will be used to statistically compare the means of off-target mutation rates from the *in vivo* and *in vitro* treatments. We do not expect to observe a significant difference in the ratio of

off-target/on-target mutations between the two treatments, as the same gRNA, donor DNA, and vectors are used. However, we may observe a reduction in the number of observed off-target mutation in the *in vivo treatment,* if there is an overall reduction of efficiency.

On-target mutations.

A quantification of how often the CRISPR complex is both cutting the correct sequence and effectively incorporating the donor DNA is vital to future claims regarding the efficiency of CRISPR and its potential clinical use. The *in vivo* and *in vitro* on-target rates will be compared via a two-tailed t-test and analyzed to determine statistically significant difference. For example, we will compare the fraction of BFP-expressing cells, GFP-expressing cells, cells lacking fluorescence between the two treatments. The on-target rates will be characterized by the appearance of BFP. Although the essential requirements for CRISPR to perform efficiently within a cell are met in both the *in vivo* and *in vitro* treatments and in theory the on-target mutation rates would not vary significantly between the two, *in vivo* delivery may pose a problem. If the CRISPR delivery or exposure is compromised via the nature of *in vivo* delivery, we could expect to see a lower on-target rate and off-target rate amongst the *in vivo* treatment. However, this result would not negate further *in vivo* genetic editing research, but could instead point to a need for further research of effective *in vivo* CRISPR complex delivery.

Overall efficiency.

While off-target rates are characterized as off-target per on-target mutation, the overall efficiency reflects the overall amount of mutations, any change at all. In regards to our fluorescence test, this would resemble any change from green to either blue or non-fluorescence. Although the off-target and on-target may not vary between the two treatments, the total efficiency should be analyzed. This information can then be useful when determining the current clinical viability of *in vivo* genetic editing of oocytes. If the efficiency is lower but the off-target rate is the same or lower, this may be interpreted as a low-risk situation.

CONCLUSION

In the first chapter of this thesis I examined current conservative Christian discourse on genetic editing and located four major Christian concerns commonly used in the Christian rejection of genetic editing research and clinical usage. These concerns (beginning of life, Creator-human relationship, imago *Dei*, and stewardship) are also tension zones within the discourse that contain contradictions and allow for the possibility of potential acceptance of genetic editing. In the second chapter, I applied this knowledge of tension zones to argue that two areas in which there is considerable possibility of Christian acceptance of genetic editing are the stewardship concern of harm and the conjugal act requirement in the creation of new life. I propose that epigenetics, somatic gene therapy and *in vivo* genetic editing are promising research fields that could help produce genetic editing methods that accommodate previously identified Christian concerns. The final chapter is an experimental proposal for an analysis of *in vivo* and *in vitro* CRISPR-Cas9 genetic editing efficiency; here efficiency is quantified by both on- and off-target mutation rates which quantify the amount of correct mutations at the targeted location and the amount of mutations at different similar-looking sites. This study is important when determining the clinical possibility of *in vivo* genetic editing and whether the risks out way the benefits.

This thesis is important as it shows that not only is it possible to study a specific discourse to determine important community concerns, but also that it is possible to scientifically pursue techniques that accommodate these concerns and allow for wider acceptance. Further studies in the three research fields I proposed

would support a greater goal of increasing the clinical accessibility of genetic editing. This is especially important for those who find themselves in situations where they would like to have the choice of genetic editing but are currently uncomfortable with contemporary methods. Not only is scientific research important in motivating this goal, but also religious studies research is important. Further analysis of other, non-majority religious discourse regarding genetic editing will help us understand their concerns and develop research paths that can help accommodate those concerns, producing clinical genetic editing methods that they would feel more comfortable using.

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