The influence of aging on oocyte quality and the importance of mitochondria

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Abstract of Thesis

Female fertility peaks at 25 years of age, yet many women put off having children until much later in life (Nybo Andersen et al. 2000; Hook 1981; Hamilton et al. 2013). Age is a major determining factor in pregnancy outcomes and increased maternal age is correlated with increased chances of aneuploidy and miscarriage (Robinson et al. 2001; Benadiva et al. 1996; Battaglia et al. 1996). When older women encounter difficulties with having a successful pregnancy, it is often due to poor oocyte quality as a result of mitochondrial dysfunction (Eichenlaub-Ritter et al. 2011; Bentov et al. 2010; Jansen & Burton 2004). Mitochondria are the energy-producing organelles of the cell and are important in producing adenosine triphosphate (ATP) to meet the high energy demands of the oocyte. Mitochondria have their own DNA (mtDNA) inherited maternally that encodes proteins necessary for oxidative phosphorylation and subsequent ATP production. Along with ATP, mitochondria also produce harmful reactive oxygen species (ROS) at a low, but significant rate (Takeo et al. 2013; Parsons et al. 1997). With natural aging, mtDNA damages due to ROS can accumulate and disrupt normal cell function and ATP production. Without adequate ATP supply, oocytes fail to fertilize, implant, or develop properly during pregnancy. This eventual decline of oocyte quality occurs with natural aging, but decreased fertility is also observed in diabetes and other metabolic disorders (Moley et al. 1991; Sadler et al. 1988). As the number of women and couples seeking fertility treatments increases, it is important to understand why pregnancy fails. In this thesis, oocyte quality and its decline with maternal age are discussed, with a focus on the effects of mitochondrial dysfunction. Factors and disorders affecting proper mitochondrial function are introduced, and methods of both improving and preserving oocyte quality are reviewed.
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>aCGH</td>
<td>array comparative genomic hybridization</td>
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<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
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<td>AGE</td>
<td>advanced glycation end-product</td>
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<tr>
<td>AL</td>
<td><em>ad-libitum</em></td>
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<tr>
<td>APC/C</td>
<td>anaphase promoting factor/cyclosome</td>
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<tr>
<td>ART</td>
<td>assisted reproductive technologies</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>AUGMENT</td>
<td>autologous germline mitochondrial energy transfer</td>
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<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CGH</td>
<td>comparative genomic hybridization</td>
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<td>CoQ10</td>
<td>coenzyme Q10</td>
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<td>CR</td>
<td>caloric restriction</td>
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<td>FADH$_2$</td>
<td>flavin adenine dinucleotide</td>
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<tr>
<td>FISH</td>
<td>fluorescence <em>in situ</em> hybridization</td>
</tr>
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<td>FSH</td>
<td>follicle stimulating hormone</td>
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<tr>
<td>H$_2$O$_2$</td>
<td>hydrogen peroxide</td>
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<tr>
<td>ICSI</td>
<td>intracytoplasmic sperm injection</td>
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<tr>
<td>IMM</td>
<td>inner mitochondrial membrane</td>
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<tr>
<td>IVF</td>
<td><em>in vitro</em> fertilization</td>
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<td>LH</td>
<td>luteinizing hormone</td>
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<tr>
<td>mΔΨ</td>
<td>mitochondrial membrane potential</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
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<tr>
<td>MCC</td>
<td>mitotic checkpoint complex</td>
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<td>MG</td>
<td>methylglyoxal</td>
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<td>mtDNA</td>
<td>mitochondrial DNA</td>
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<td>MTOC</td>
<td>microtubule-organizing center</td>
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<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
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<td>OMM</td>
<td>outer mitochondrial membrane</td>
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<tr>
<td>OSC</td>
<td>oogonial stem cell</td>
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<tr>
<td>PCOS</td>
<td>polycystic ovarian syndrome</td>
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<tr>
<td>PI3K</td>
<td>phosphoinositide 3-kinase</td>
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<tr>
<td>POLγ</td>
<td>DNA polymerase gamma</td>
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<tr>
<td>PSSC</td>
<td>premature separation of sister chromatids</td>
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<tr>
<td>RCS</td>
<td>reactive carbonyl species</td>
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<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<td>ROS</td>
<td>reactive oxygen species</td>
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<td>rRNA</td>
<td>ribosomal RNA</td>
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<tr>
<td>SAC</td>
<td>spindle assembly checkpoint</td>
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<td>SIRT1</td>
<td>sirtuin 1</td>
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<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
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<tr>
<td>TCA</td>
<td>tricarboxylic acid</td>
</tr>
<tr>
<td>TFAM</td>
<td>mitochondrial transcription factor A</td>
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<td>tRNA</td>
<td>transfer RNA</td>
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Introduction

In today’s society, women are deciding to have children later than ever before. A record number of women in their 20’s are putting off having families until later in life, and many are choosing to start careers before having children (Hamilton et al. 2013). While the birth rates for women in their 20’s were reported at a record low of 83.1 births per 1,000 women in 2012, birth rates for women in their 30’s and 40’s have increased (Hamilton et al. 2013). Couples of all ages seek fertility assistance for a number of reasons, but younger women are more likely to have successful pregnancies on their own (Van Voorhis 2006). Women in their 20’s have approximately a 9% chance of suffering from miscarriage at some point during pregnancy, but this number jumps to 75% for women 40 years old and above (Nybo Andersen et al. 2000). This has led to an increase in the number of couples and individuals seeking fertility assistance, and sparks the conversation about the negative relationship between oocyte quality and maternal age.

It is well known that as a woman matures, her chances of successful pregnancy decrease. Although women in their 40’s and 50’s have been known to have healthy pregnancies, female fertility typically peaks at age 25 years and drops dramatically after age 35 (Nybo Andersen et al. 2000; Hook 1981).

Oocyte quality plays an important role in the determination of pregnancy success. Though assisted reproductive technology (ART) methods often fail for older women using their own oocytes, success can be achieved using donor oocytes from younger women, and post-menopausal women have also served as successful surrogates (Sauer et al. 1995). This demonstrates that decreased fertility appears to lie majorly within the oocyte itself rather than the uterine environment. An increased understanding of the effects of aging and other metabolic disorders on oocyte quality would give researchers and clinicians the ability to improve fertility
and pregnancy outcomes for many women. While many technologies have been developed to increase the chances of pregnancy for patients, the efficiency rates of these methods are still low and there is much room for improvement (CDC 2013). To increase egg quality in both ART and natural conception, it is important to understand what exactly defines a “good” oocyte and the mechanisms behind its ultimate failure.

Many mechanisms of fertilization and embryogenesis fail when oocyte quality is poor. Depending on the degree of impairment, failure can occur during fertilization, implantation into the uterine wall, or later in development. Decreased oocyte quality often results in aneuploidy, the incomplete separation of chromosomes during meiosis. When this occurs, cells contain the incorrect number of chromosomes needed for proper development. Aneuploidy can occur in any of the 23 pairs of chromosomes, but most aneuploid embryos do not survive. In fact, euploid embryos are much more likely to develop to the blastocyst stage than aneuploid embryos (93% vs. 21%) (Sher et al. 2007). Trisomies, a result of aneuploidy, are more often seen in pregnancies of older women than younger women. The chances of a woman giving birth to a child with trisomy 21, also known as Down syndrome, increase to 1 in 11 at age 49 (Hook 1981).

Aneuploidy and other problems that arise with advanced maternal age are associated with an increase in the degree of mitochondrial dysfunction present in oocytes (Volarcik et al. 1998). As a woman ages, oocyte quality declines until eventual ovarian failure, also known as menopause. When oocytes begin to “go bad”, it is often due to accumulated mitochondrial dysfunction (Chistiakov et al. 2014; Jansen & Burton 2004; Bentov et al. 2011; Kushnir et al. 2012). As a result, oocytes do not have enough energy available to perform necessary cellular functions. This is disadvantageous for oocytes, since spindle formation and chromosomal
segregation, as well as other important stages of cell division, are highly energy-intensive processes (reviewed in Dumont & Desai, 2012).

Mitochondria are the organelles within the cell that supply most of the necessary energy needed for proper function in the form of adenosine triphosphate (ATP), and play a crucial role in metabolism. Mitochondria contain their own DNA separate from nuclear DNA, and have approximately 1-10 copies of circular mitochondrial DNA (mtDNA) per organelle (Anderson et al. 1981). The 13 proteins encoded by mtDNA make up many of the electron transport chain complexes needed for oxidative phosphorylation, and additional proteins necessary for mitochondrial function are translocated from the nucleus (Schatz 1979). ATP is created through the process of oxidative phosphorylation carried out across the electron transport chain within the inner membrane of the mitochondria. Oxidative phosphorylation typically results in the formation of ATP and water, but this process is not 100% efficient (Porter & Brand 1995). When oxygen, the final electron acceptor, is incompletely reduced, superoxide radicals are formed. Superoxide radicals can form reactive oxygen species (ROS) that disrupt normal cellular activities and damage DNA molecules when not balanced with antioxidant defenses (Halliwell 2007; Raha et al. 2000; Haigis & Yankner 2010). To compound the problem, dysfunctional or damaged mitochondria produce increased levels of ROS, thereby damaging more mitochondria in the process (Crompton 1999). MtDNA molecules are in such close proximity to the production of ROS within the mitochondria that they are unable to avoid damage. The nucleus employs certain protective mechanisms to preserve the integrity of nuclear DNA from ROS-induced oxidative damage, but these are lacking in mtDNA. Unlike nuclear DNA, mtDNA is not wound around protective histone proteins, contains no non-coding introns,
and lacks the repair mechanisms necessary to repair damage caused by ROS (reviewed in Palikaras & Tavernarakis, 2014). This leaves mtDNA highly susceptible to mutations.

Whereas nuclear DNA is inherited from both parental lineages, and therefore able to overcome genetic mutations to a certain degree, mtDNA is uniparental and only inherited from the mother (Giles et al. 1980). Sperm mitochondria are destroyed shortly after fertilization by autophagy (Kaneda et al. 1995; Thompson et al. 2003). For this reason, it is remarkable that a woman of 25 years, whose oocytes have presumably experienced 25 years of ROS exposure, can give birth to healthy children at a high rate of success (Hamilton et al. 2013). Because mtDNA inheritance is uniparental, it is important to consider the degree of fertility of children born to older mothers. The age of the maternal grandmother at the time of the mother’s birth is an influential factor associated with an increased risk of Down syndrome (Malini & Ramachandra 2006; Aagesen et al. 1984). For every year after age 30, the risk of the grandchild having Down syndrome increases by 30% (Malini & Ramachandra 2006; Aagesen et al. 1984).

Mitochondrial dysfunction can be measured in a number of ways. MtDNA copy number can be used to predict which oocytes are more likely to complete successful fertilization and embryogenesis through development (Wai et al. 2010). A threshold (about 50,000 copies in both humans and mice) exists for the amount of mtDNA necessary for development, and oocytes with an mtDNA copy number below the threshold are less likely to implant into the uterine wall during blastocyst formation (Wai et al. 2010). From fertilization until implantation, mtDNA replication is halted in mammalian zygotes. However, the early embryo is still growing and developing at this time, and rapid cell division causes a dilution effect of mtDNA within individual cells until replication can be restarted. If the original oocyte has mtDNA copy
numbers below the threshold, it is not able to recover from this rapid cell division in order to continue embryogenesis (Wai et al. 2010).

The integrity of mtDNA is also important for normal mitochondrial function. When mtDNA becomes damaged, the mitochondrion tries to rescue itself by fusing with another healthy mitochondrion. This dilutes the defective mtDNA with healthier mtDNA and can ameliorate its effects on the cell. If the damage is beyond repair or accumulates to an irreparable degree, the mitochondrion is phagocytized through a process called mitophagy that clears the damaged mitochondrion from the cell (Lemasters 2005). With age, this clearance mechanism becomes impaired and increased numbers of dysfunctional mitochondria begin to aggregate within cells (Wilding et al. 2001). As dysfunctional mitochondria aggregate, increased levels of ROS are produced and damage is often compounded.

DNA polymerase-γ (POLγ) is an enzyme translocated from the nucleus that is responsible for replicating mtDNA within mitochondria. Because POLγ is the only DNA polymerase within mitochondria (Bebenek & Kunkel 2004), any mutations in the catalytic subunits may lead to dysfunction of replication. Mitochondrial dysfunction can occur from a number of mutations in Polγ, causing decreased oocyte quality and mitochondrial diseases in mutant mice (Chan & Copeland 2009).

Mitochondria contain both an inner and outer membrane, both of which are important for functional oxidative phosphorylation. Maintenance of mitochondrial membrane potential is correlated with the ability of the organelle to effectively produce ATP (Wilding et al. 2003; Van Blerkom et al. 1995). Embryos with lower membrane potential are more likely to have random segregation of chromosomes (Wilding et al. 2003). If the mitochondrial membrane potential is decreased or disrupted, mitochondria lose the ability to create the electron gradients necessary to
drive ATP production. Therefore, mitochondrial membrane potential is typically used as an indicator of mitochondrial dysfunction (Komatsu et al. 2014; Wilding et al. 2003).

It is of great importance to understand why oocytes fail and how it may be possible to reverse their phenotypes to resemble younger, healthier oocytes. Diabetic or obese women often have oocytes with similar phenotypes as older women, regardless of their age, and therefore have an increased risk of aneuploidy and miscarriage (Sadler et al. 1988; Colton et al. 2002). Many women also suffer from premature ovarian failure (menopause onset before age 40) for a number of reasons, including genetic disorders like Turner syndrome, or chemotherapy and other cancer treatments (Kalantaridou et al. 1998). Women with these conditions, as well as those with fertility lost by natural aging, would certainly benefit from treatments aimed at increasing oocyte quality.

While many improvements have been made in the last few decades in the field of assisted reproduction, no method has been able to achieve 100% success. The successful use of young donor eggs for aged women, or older surrogates for younger women with in vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI) demonstrate that oocyte quality is more of a determinant of pregnancy success than the receptivity of the host uterus; older patients using oocytes donated from younger patients are able to carry successful pregnancies when using their own aged oocytes fails (Sauer et al. 1995). With this knowledge, clinicians took a small amount of cytoplasm from a young oocyte and transferred it into an oocyte of patient with previously failed pregnancy attempts in the hopes of improving oocyte quality. Successful pregnancies were achieved with this method (Cohen et al. 1998), but it was soon realized that these children had inherited mtDNA from both the mother and the donor, resulting in mitochondrial heteroplasmy (Brenner et al. 2000; Barritt et al. 2001). Because the long-term effects of this
condition are unknown, the US Food and Drug Administration put a moratorium on this procedure in 2001. For this reason, cytoplasmic transfers are not a feasible method for improving oocyte quality. However, it is generally accepted that the mitochondria within the young oocyte are the rejuvenating factor within the cytoplasm. Therefore, improving mitochondrial function to increase oocyte quality has become a recent focus of research.

One such improvement has been the use of autologous, healthy mitochondria from a woman’s oogonial stem cells to increase the quality of her oocytes during ART procedures (Woods et al. 2013). Oogonial stem cells are adult stem cells that produce new immature oocytes postnatally in mammalian females (Johnson et al. 2004). Mitochondria within these oogonial stem cells, which are from the same cell lineage as oocytes, would therefore provide the best autologous source of mitochondria for improving oocyte quality in patients. These oogonial stem cell mitochondria are less differentiated than oocytes residing in the ovary, and therefore are less likely to have accumulated genomic damage that would cause dysfunction. The process, coined “AUGMENT” (autologous germline mitochondrial energy transfer), is promising (reviewed in Tilly & Sinclair, 2013), but extracting oogonial stem cells from female patients and further isolating mitochondria is challenging and invasive. Therefore, emphasis has shifted from transferring functional mitochondria into poor-quality oocytes to how to improve a woman’s endogenous oocyte mitochondria.

Because mitochondrial function is impaired mostly by ROS, scientists have begun to focus on how to counteract this damage. Antioxidant treatments have been promising as a way to preserve oocyte quality in aged females, but can cause additional damage to the function of reproductive somatic tissues in the process, thereby decreasing overall fertility (J J Tarín et al. 2002). Levels of the necessary electron transport chain component Coenzyme Q10 (CoQ10)
naturally decrease with age (Pignatti et al. 1980), which once again links aging with increased mitochondrial dysfunction. CoQ10 supplements have been shown to increase oocyte quality and menopausal cognitive effects in mice by affecting mitochondrial dysfunction and ROS production (Gendelman & Roth 2012; Sandhir et al. 2014). Resveratrol, a dietary-restriction mimetic, has also been suggested to improve oocyte quality by acting as a potent antioxidant. This polyphenol has been shown to preserve oocyte quality in aging mice (Liu et al. 2013) as well as improve anti-oxidation levels in bovine oocytes and embryos (Takeo et al. 2013). Resveratrol acts through sirtuin 1 (SIRT1) to reduce oxidative stress by increasing mitochondrial number and function, and SIRT1 inhibitors cause decreased fertilization (Takeo et al. 2013; Takeo et al. 2014).

Another approach to preserving oocyte quality with age in mice is caloric restriction. When diets are restricted, fertility is reduced, but it is able to rebound once normal feeding is resumed (Ball et al. 1947; Nelson et al. 1985; Visscher et al. 1952; Lintern-Moore & Everitt 1978). When applied to aged mice with poor oocyte quality, this treatment resulted in marked increases in the fertility of females compared to age-matched controls once the dietary restrictions were removed (Selesniemi et al. 2008). Because obesity and diabetes are linked with subfertility in women of any age (Purcell & Moley 2011; Wang & Moley 2010; Grindler & Moley 2013; Jungheim & Moley 2010), it is not surprising that oocyte quality benefits from a low-calorie diet. Because similar studies have yet to be conducted in humans, it is unclear whether the treatment durations could be feasibly translated from mice to women.

Summary
Regardless of the underlying mechanisms, it is clear that female fertility eventually reaches its limit by the fifth or sixth decade of life (Richardson et al. 1987). Aged oocytes have different characteristics from younger oocytes, and understanding what makes them so different may provide clues for improving quality. Oocytes from young, healthy females have a stable mitochondrial membrane potential, mtDNA copy numbers well above threshold, and mtDNA with minimal damage. Due to efficiently functioning mitochondria, adequate levels of ATP are provided to the cell, allowing for normal spindle formation and equal chromosomal segregation during meiosis. In contrast, poor quality oocytes show a reduced mitochondrial membrane potential, mtDNA copy number, and ATP levels, often due to (or resulting in) increased mtDNA damage. Because ATP production is disrupted, chances of aneuploidy and developmental failure are increased. Methods to improve oocyte quality have been developed, but a single method that achieves 100% success has not yet been discovered. A better understanding of the mechanisms responsible for oocyte failure is clearly required to move forward in the field of ART and fertility treatments.

This thesis will review the present literature on oocyte quality and how it is negatively affected by age, with a focus on mitochondrial dysfunction. Factors that determine oocyte quality, the consequences of poor quality, and current methods used to improve quality will also be discussed.
CHAPTER 1: Oocyte development and maturation

It has long been believed that women are born with all of the oocytes they will ever have throughout their lifetime (Zuckerman 1951). Because menopause, caused by reduced ovarian function and eventual ovarian failure, occurs naturally with age, it was previously assumed that menopause is the result of a depletion of healthy ovarian follicles (Richardson et al. 1987). Many suggested that once a woman’s follicular reserve she was endowed with at birth was exhausted, the ovaries were depleted and menopause was initiated (Richardson et al. 1987). For decades this dogma remained unchallenged, yet females of many other vertebrate and invertebrate species studied exhibit some form of germline stem cell and produce new oocytes throughout adulthood (Kimble 1981; Hirsh et al. 1976; Lin & Spradling 1997; Nakamura et al. 2010), making mammals the exception to the rule.

In 2004, it was discovered that mitotic germ line stem cells are present within the ovaries of adult female mice (Johnson et al. 2004). These oogonal stem cells have been isolated from mouse ovaries and used to produce live offspring (Zou et al. 2009). Additionally, oogonal stem cells have been found in the ovaries of adult women (White et al. 2012), suggesting that ongoing germ cell renewal can occur in humans of both sexes. It is currently unclear the extent of which these cells can be used to improve female fertility, but many studies have suggested using these undifferentiated cells as reproductive therapies and possibly replacement oocytes. It has been suggested that older women whose mature oocytes are damaged from aging may benefit from the use of autologous oogonal stem cell replacements (reviewed in Tilly & Sinclair 2013). While many therapies are currently practiced for assisted reproduction, this recent discovery reinforces the notion that there is still much to be learned about female reproduction.
Development of primordial follicles to fertilization of the oocyte

The generation of oocytes first begins in the fetal ovary. Mitotic divisions increase the number of germ cells within the fetal ovary, and incomplete cytokinesis creates the formation of joined cells known as germ cell cysts (Pepling & Spradling 2001; Kezele et al. 2002). At approximately the 11th or 12th week of gestation, follicles begin to form and meiosis begins (Gondos et al. 1986; Martins da Silva et al. 2004). It has been suggested that retinoic acid triggers the switch from mitosis to meiosis that defines the transition from oogonia to oocytes (Bowles & Koopman 2007). This switch causes the germ cell cysts to dissociate and individual oocytes become surrounded by a single layer of pre-granulosa somatic cells to form primordial follicles (Kezele et al. 2002).

Before arresting in prophase I, oocytes must carry out synapsis and recombination of homologous chromosomes to ensure successful future development and survival (reviewed in Eichenlaub-Ritter, 1998). The location and number of crossovers during meiosis I are important for preventing errors in the first meiotic division (reviewed in Hunt & Hassold, 2008). Crossovers too close to either the telomeres or centromeres of the chromosomes can have detrimental effects on future embryonic development and survival (Hassold et al. 2007). During follicular formation, gap junctions are created between follicular somatic cells and the oocyte in order to coordinate their functions. Atresia, a form of regulated cell death, occurs continuously throughout follicular development and the majority of follicles die before ovulation (reviewed in Hunt & Hassold, 2008; Martins da Silva et al., 2004). Those that do make it move on to the later stages of follicular development. Oocytes within primordial follicles continue through meiosis to prophase I, where cell division is arrested in the dictyate stage. Although it is unknown what
causes the recruitment of follicles from the primordial pool, many primordial follicles remain dormant in humans for decades before further development and ovulation occur.

Once follicles are recruited from the primordial follicle pool, follicular development resumes while the oocyte remains in prophase I. As the primordial follicles develop into primary follicles, the granulosa cells become more cuboidal and a theca layer develops (Kezele et al. 2002). Granulosa cells are in direct contact with the oocyte, whereas theca cells form in the surrounding stromal tissue. Gap junctions between somatic cells and also between somatic cells and the oocyte are important during early follicular growth, as gonadotropin levels that support development are only adequate after reaching sexual maturation (i.e. puberty) (Hutt & Albertini 2007; Downs 1995; Donahue & Stern 1968; Grazul-Bilska et al. 1997). Both the surrounding somatic cells and oocytes rely on each other for bi-directional signaling to promote maturation and development of the follicle (Knight & Glister 2006; Hutt & Albertini 2007; Woodruff & Mayo 2005; Kezele et al. 2002). As the follicle grows, the number of cuboidal granulosa cell layers increases and the oocyte grows larger. Once the follicle has developed to the pre-antral stage, follicular fluid begins to form between the granulosa and theca cell layers (Hirshfield, 1991; reviewed in Peters, 1969). When a woman enters puberty, the oocyte is now exposed to gonadotropins such as follicle stimulating hormone (FSH) that support the growth and ovulation of large, Graafian follicles (Halpin et al. 1986; Cattanach et al. 1977; Kumar et al. 1997). This transition is significant, as development from a pre-antral to antral follicle provides the oocyte with the ability to resume meiosis (Sorensen & Wassarman 1976).

Mid-way through the menstrual cycle, the surge of luteinizing hormone (LH) induces the completion of meiosis I and the ovulation of the mature oocyte from the ovary. Whereas oocytes arrested in prophase I are characterized by an intact nuclear membrane known as the germinal
vesicle, oocytes that have completed meiosis I show germinal vesicle breakdown, condensation of chromosomes, and the assembly of the bipolar spindle (Leung & Adahshi 2004). The completion of meiosis I segregates homologous chromosomes into the oocyte and the first polar body. After meiosis I, the oocyte becomes arrested again, this time in metaphase II with sister chromatids aligned on the metaphase II plate. Meiosis II is completed and the second polar body is formed once fertilization occurs. If fertilization is successful, pronuclei form around the genetic material of the oocyte and the sperm. The first mitotic division is initiated once the pronuclear envelopes dissolve and genetic material combine to form the zygote.

**Spindle assembly in oocytes**

In order for cell division to occur, oocytes must have proper spindle formation. Mitotic somatic cells contain centrosomes from which the centrioles are formed on either side of the cell. These centrioles assemble alpha and beta tubulin to make microtubules of the spindle (Vogt et al. 2008; Dumont & Desai 2012). These microtubules then extend towards the center of the cell, continuously lengthening and shortening in attempts to attach to the kinetochores of chromosomes. Once chromosomes are properly aligned and the correct connections are made with microtubules, the cell proceeds into anaphase where the chromosomes are pulled apart from their kinetochores and are equally separated into two daughter cells (Vogt et al. 2008; Dumont & Desai 2012).

Cell division proceeds in a similar fashion in sperm, but oocytes have a slightly different process. Oocytes lack centrosomes and therefore meiosis in oocytes is considered an “acentrosomal” process. Rather than originating on the periphery of the cell, microtubule-organizing centers (MTOCs) are adjacent to chromosomes throughout the cell. Once meiosis is
initiated, the MTOCs form a ball in the center of the cell and the chromosomes line up along its center during metaphase. Microtubules must make proper connections with chromosomes to enter into anaphase, although the checkpoint here is not as strict as it is in somatic cells and sperm cells (LeMaire-Adkins et al. 1997). As microtubules are pulled towards opposing sides of the cell, the chromosomes are pulled along with them, segregating the chromosome pairs into two cells.
CHAPTER 2: Mitochondrial function and structure

Mitochondria are the major energy producers of eukaryotic cells. These organelles are the site of many metabolic functions including the tricarboxylic acid (TCA) cycle (also known as the Krebs cycle or the citric acid cycle) and oxidative phosphorylation, which lead to ATP production. ATP is used by countless energy-dependent processes in the cell including cell division, proteasome activity, and microtubule function (Goldberg & St John 1976; Bershadsky & Gelfand 1981; Gottesman & Maurizi 1992; Robinson & Spudich 2000). Mitochondria are made up of two membranes, the inner mitochondrial membrane (IMM) and the outer mitochondrial membrane (OMM). The IMM is made up of many folded cristae, which increase the surface area of the IMM to allow for additional production of ATP. Within the IMM lies the mitochondrial matrix, the site of the TCA cycle. The electron transport chain is made up of various protein complexes located across the IMM that produce a proton gradient as a result of the transfer of electrons between complexes. Mitochondria then utilize this proton gradient created within the intermembrane space to drive ATP production (reviewed in Lodish, Berk, & Zipursky, 2000).

Mitochondrial DNA

As a result of the endosymbiosis of two prokaryotic cells that formed the first eukaryotic cell, mitochondria contain approximately 1-10 copies of their own DNA distinct from the cell’s nuclear DNA (Anderson et al. 1981). The importance of mitochondria in oocyte quality stems from the fact that mitochondrial DNA (mtDNA) is maternally inherited, rather than nuclear DNA, which is inherited as a combination of maternal and paternal DNA (Giles et al. 1980). Although it is unclear exactly how the mechanism functions, it is known that upon fertilization,
paternal mtDNA is degraded by ubiquitin-proteasome machinery within the oocyte that recognizes the sperm cell’s ubiquitin substrates, targeting the mitochondria for destruction (Kaneda et al. 1995; Thompson et al. 2003). Mitochondrial DNA molecules from the mother then go on to act as a template for all of the mtDNA found in the offspring. This 16.5kb circular DNA strand contains 37 genes that encode 13 proteins, 22 transfer RNAs (tRNAs), and 2 ribosomal RNAs (rRNAs) (Anderson et al. 1981). Proteins encoded by mtDNA include subunits of the electron transport chain that are essential for ATP production. Although mtDNA only codes for 13 proteins, there are over 1500 found in mitochondria, indicating that the remaining proteins are imported from the nucleus (Schatz 1979). Proteins found in the mitochondria are specific to the cell type, which demonstrates cross-talk between mitochondria and nuclei to carry out specific cell functions (Johnson et al. 2007).

mtDNA replication

In order for mtDNA to be successfully transmitted from mother to offspring, it must undergo DNA replication at the appropriate time during development. mtDNA molecules contain a heavy and a light strand that must both be replicated (reviewed in Shoubridge & Wai 2007). There are multiple theories on how exactly the circular mtDNA is replicated, two of which being the coupled leading/lagging strand synthesis model and the asymmetrical synthesis model (Yasukawa et al. 2005; Shadel & Clayton 1997). The coupled leading/lagging strand model predicts that both the heavy and light strands are replicated simultaneously in opposite directions from the same origin (Yasukawa et al. 2005). On the other hand, the asymmetric synthesis model states that the heavy strand is synthesized first, and the light strand initiates replication when the heavy strand replication has reached two thirds of completion (Shadel &
Clayton 1997). Unfortunately conclusive evidence is lacking to determine which model is accurate, and many scientists disagree (Bogenhagen & Clayton 2003; Holt & Jacobs 2003).

Although oocytes contain more mitochondria per cell than any other cell type, the immature mitochondria of oocytes and early embryos are rounded with few cristae and little capacity for oxidative phosphorylation (Sathananthan et al. 2002; P May-Panloup et al. 2005; Pikó & Matsumoto 1976; Jansen & de Boer 1998). It is not until embryonic cells begin to develop that mitochondria become elongated and gain the ability to carry out typical metabolic function (Trimarchi et al. 2000; Wilding et al. 2001; Houghton 2006). Until blastocyst implantation, mtDNA replication is arrested and the expression of replication factors is extremely low (Thundathil et al. 2005; Pascale May-Panloup et al. 2005; Spikings et al. 2007; Pikó & Taylor 1987). MtDNA copy number decreases due to increased cellular division during these stages of embryogenesis, further reducing the mitochondrial capacity of each cell to carry out oxidative phosphorylation (Thundathil et al. 2005; Pikó & Taylor 1987). Replication of mtDNA begins again when the blastocyst implants into the uterine wall and replication factors are once again upregulated (Spikings et al. 2007; Thundathil et al. 2005; Pascale May-Panloup et al. 2005; Pikó & Taylor 1987). This process of cell division without mtDNA replication followed by the replication of the remaining mtDNA molecules is thought to create genetic drift among mtDNA variants in attempts to prevent mutant mtDNA from populating the offspring (Fan et al. 2008; Stewart et al. 2008). This creates a mitochondrial bottleneck through which the random selection of mtDNA occurs (Hauswirth & Laipis 1982). Though the details of this selection process are unclear, it may not be entirely random, as large mtDNA mutations are typically not passed onto offspring as frequently as would be expected by chance (Keefe et al. 1995).
Metabolism – electron transport chain and oxidative phosphorylation

One of the most important roles of mitochondria is their function in cellular metabolism. Many species use aerobic cellular respiration to convert glucose into ATP energy through glycolysis, the TCA cycle, and oxidative phosphorylation (reviewed in Karp, 2013; Lodish, Berk, & Zipursky, 2000). Glucose is first converted into pyruvate through glycolysis in the cytoplasm of the cell and then to acetyl-CoA by pyruvate dehydrogenase (reviewed in Balaban et al. 2005). Products of the TCA cycle such as NADH and FADH$_2$ become oxidized and donate electrons to the first steps of the electron transport chain to initiate oxidative phosphorylation. Oxidative phosphorylation involves the transfer of electrons along a chain of proteins that subsequently uses redox reactions to create a proton gradient across the IMM. This built up gradient of protons then drives the final enzyme in the electron transport chain, ATP synthase. When protons flow back down the generated concentration gradient and return to the mitochondrial matrix, it drives the phosphorylation of adenosine diphosphate (ADP) by ATP synthase to create ATP. Oxidative phosphorylation produces approximately 34 ATP per glucose molecule, whereas aerobic respiration as a whole generates up to 38 ATP total (Rich 2003; Boyer et al. 1977; Lodish et al. 2000).

Complex I, NADH dehydrogenase, is the first enzyme in the electron transport chain, and is made up of proteins encoded by both nuclear and mitochondrial DNA. When NADH is hydrolyzed into NAD$^+$ and a proton, 2 electrons are donated to complex I, which drives 4 protons across the IMM into the intermembrane space. Complex II, succinate dehydrogenase, acts as a second point of entry for electron donation and, like complex I, links oxidative phosphorylation with the TCA cycle by oxidizing FADH$_2$. This enzyme oxidizes succinate to
fumarate and reduces ubiquinone, which does not create as much energy as complex I, and therefore does not contribute to the proton gradient across the IMM (Lodish et al. 2000; Karp 2013). Electrons from both complexes I and II are further transferred one at a time to coenzyme Q10, also known as ubiquinone. This carrier then passes the electrons onto complex III, coenzyme Q reductase, where 4 protons are transferred across the IMM into the intermembrane space. From complex III, electrons are passed onto cytochrome c, which acts as a substrate for complex IV. The final electron acceptor in the electron transport chain is oxygen, which produces two water molecules as final end products of oxidative phosphorylation after receiving 4 electrons from complex IV. This reduction releases two final protons across the IMM to add to the electrochemical gradient. Therefore, a total of 10 protons are released into the intermembrane space for each pair of electrons transferred through oxidative phosphorylation to create a concentration gradient across the IMM (Lodish et al. 2000). ADP is phosphorylated to ATP when protons move down their electrochemical gradient to drive the function of ATP synthase. ATP synthase is made up of two joined complexes, a rotating hexamer driven by a transmembrane proton channel, which function together to drive ATP synthesis.

**Reactive oxygen species**

Although oxidative phosphorylation is important for cell survival, it is not 100% efficient (Porter & Brand 1995). Electrons can leak out of the electron transport chain when they are not sufficiently transferred between the complexes and can react with oxygen to create superoxide radicals (O$_2^-$). Complexes I and III are known to be the main source of superoxide radicals in mitochondria (Dröse & Brandt 2012; Lesnefsky et al. 2001; Lenaz 2012; Sugioka et al. 1988; Wallace 2000). ROS are created when these superoxide radicals are not converted into hydrogen.
peroxide (H$_2$O$_2$) or water via the antioxidant enzyme superoxide dismutase (SOD) (Halliwell 2007; Raha et al. 2000). While ROS are typically known as damaging molecules, they have also been found to play a role in normal cell function at low levels by acting as second messengers in MAPK and PI3K pathways, as well as the immune response (Devadas et al. 2002; Genestra 2007; Kwon et al. 2004; S.-R. Lee et al. 2002; Seo et al. 2005; Tobiume et al. 2001).

Although antioxidant defenses are in place to maintain homeostasis within the cell (Chatterjee et al. 2007; Valko et al. 2006), increases in ROS production can over-power these protection mechanisms, a process known as oxidative stress that causes cellular damage (Haigis & Yankner 2010). Increased oxidative stress can damage proteins, lipids, and nucleic acids within the vicinity of ROS production; therefore mitochondria are often the most affected organelles (reviewed in Ray, Huang, & Tsuji, 2012). When too much oxidative stress occurs, cells become unable to function (Genestra 2007). One facet of cellular aging has been attributed to the accumulation of ROS in cells and the disruption of the oxidant/antioxidant balance (Herrero & Barja 1997; Moghaddas et al. 2003). Therefore, it is important to understand how the function of mitochondria influences oocyte quality with increased maternal age, and more importantly, how oocyte quality can possibly be improved.
CHAPTER 3: Mitochondrial dysfunction

With advanced age, mitochondrial dysfunction increases in both somatic and germline cells (Taylor et al. 2003; Michikawa 1999; Kang & Hamasaki 2005; Jansen & Burton 2004; Tatone et al. 2010; Eichenlaub-Ritter et al. 2011). As cells age through natural processes, the ability to produce ATP is gradually lost through several mechanisms, including decreased mitochondrial membrane potential, decreased mtDNA copy number, and increased organelle swelling (Wilding et al. 2003; De Boer et al. 1999; Müller-Höcker et al. 1996; Van Blerkom 2004). These phenomena contribute to impaired ATP production and reduced energy to carry out basic cellular functions. Oocytes are perhaps the cells most affected by mitochondrial dysfunction, as they have more mitochondria than somatic cells and provide the template for all mitochondria within offspring (May-Panloup et al. 2007; Ashley et al. 1989).

The loss of normal mitochondrial function in oocytes is often a major determinant of pregnancy failure in older women (Lee et al. 2014; May-Panloup et al. 2007; P May-Panloup et al. 2005; Wilding et al. 2009; Jansen & Burton 2004; Komatsu et al. 2014; Van Voorhis 2006). Because oocyte mitochondria provide the mtDNA for the entire offspring, healthy inherited mitochondria are important for the survival of the embryo as well as for the health of the future child (Giles et al. 1980). Without the preservation of inherited mtDNA, electron transport chain proteins important for ATP production may be transcribed incorrectly in the offspring (Preston et al. 2008). Mitochondria are the main producers of cellular ATP and are therefore important for highly energy-dependent processes such as oogenesis and embryonic development (Van Blerkom et al. 1995; Van Blerkom 2004; Wilding et al. 2009; El Shourbagy et al. 2006; Spikings et al. 2007). This chapter will examine the factors involved in mitochondrial dysfunction and how these abnormalities increase with advanced maternal age in the oocyte. Overall, many of
these factors lead to ATP depletion, which decreases the ability of oocyte fertilization, implantation, and further embryogenesis to occur successfully (Van Blerkom et al. 1995).

**Mitochondrial biogenesis vs. mitophagy**

In order to regulate mitochondrial health, there must be a balance between the creation of new mitochondria and the disposal of dysfunctional mitochondria. Mitochondrial biogenesis and mitophagy, the processes by which mitochondria are created and removed, respectively, achieve this homeostasis (Ding & Yin 2012). Mitochondrial biogenesis involves the generation of new mitochondria, and is regulated by factors such as the cellular environment (i.e. available nutrients and temperature), hormone stimulation, and growth factors (reviewed in Palikaras & Tavernarakis, 2014). When mitochondria become damaged or impaired, they fuse with other healthy mitochondria to alleviate any mtDNA damages with intact mtDNA of the healthy mitochondrion (Lemasters 2005). If mitochondria become damaged beyond the point of fusion rescue, they must be removed from the cell to prevent further harm to the surrounding healthy mitochondria, especially when this damage causes increased ROS production (Amicarelli et al. 2003; Wang et al. 2013; Takeo et al. 2013). During mitophagy, mitochondria are tagged for disposal and phagocytized by lysosomes to digest dysfunctional mitochondria (Ding & Yin, 2012; reviewed in Palikaras & Tavernarakis, 2014). The PINK1/Parkin pathway mediates mitophagy (Lazarou et al. 2012; Narendra et al. 2008). When mitochondria are dysfunctional, the PINK1 enzyme becomes stabilized on the OMM and recruits Parkin (Narendra et al. 2010). This ligase then ubiquitylates additional proteins on the OMM that allow the mitochondria to be recognized by autophagosomic machinery within the cell. The mitochondria are then
sequestered into autophagosomes that deliver the damaged organelles to lysosomes that degrade and dispose of the mitochondria (Yoshii et al. 2011; Chan et al. 2011).

This balance of mitochondrial biogenesis and mitophagy keeps cells functioning properly (reviewed in Palikaras & Tavernarakis 2014). However, with age, these processes begin to breakdown and homeostasis can be lost (FIGURE 1) (Fan et al. 2008; Bereiter-Hahn et al. 2008; Preston et al. 2008). Increased activity of mitophagy mechanisms decreases mitochondrial numbers, which lowers the overall amount of available ATP and can over-stress the remaining mitochondrial population (Dagda et al. 2008; Yan et al. 2012; Zhu et al. 2007). If the cell is unable to recover, lack of abundant ATP can ultimately lead to cellular death. However, insufficient levels of mitophagy can also have detrimental effects. When mitophagy is impaired, mitochondria begin to aggregate, which increases the amount of damaged mitochondria and also increased ROS production, further damaging the surrounding mitochondria (Cavallini et al. 2007; Masiero & Sandri 2010; Wilding et al. 2001). Accumulation of mitochondria with age is present in many organisms (H.-C. Lee et al. 2002; T.-M. Lee et al. 2002; Preston et al. 2008; Bereiter-Hahn et al. 2008), yet it is unknown whether it is caused by decreased mitophagy or increased mitochondrial biogenesis (Van Blerkom 2004).

**Mitochondrial morphology, size, and structures**

Studies from human oocytes show that both mitochondrial volume and number increase with age (Müller-Höcker et al. 1996; Steuerwald et al. 2000). In metaphase II oocytes of women aged 27 to 39 years, increased age is associated with increases in quantity, volume, and area of oocyte mitochondria (Müller-Höcker et al. 1996). Differences in mitochondrial profile area with age are most apparent when subjects are separated into two age groups: 27-30 years old, and 31-
39 years old. Aging has also been linked to morphological changes in mitochondrial cristae, membrane, and vacuole structure (Simsek-Duran et al. 2013). Metaphase II oocytes from hamsters and mice show that mitochondrial structure is significantly altered in aged oocytes from each species (Simsek-Duran et al. 2013). In addition to collapsed cytoplasmic lamellae and abnormal cristae, aged oocyte mitochondria display decreased ATP production and decreased mtDNA copy numbers (Simsek-Duran et al. 2013), suggesting that abnormal mitochondrial morphology leads to impairment of mitochondrial function. It has been proposed that increases in the overall mitochondrial volume and number are associated with a compensatory mechanism in order to “rescue” the cell from decreased mitochondrial function with age (Müller-Höcker et al. 1996). When additional mitochondria become dysfunctional and are not disposed of by mitophagy, the cell attempts to maintain ATP production by increasing the size and number of the remaining mitochondria (Müller-Höcker et al. 1996). However, if these remaining mitochondria are already dysfunctional, increasing the existing mitochondrial profile only exacerbates present issues, leading to further mitochondrial dysfunction and oocyte failure (Wilding et al. 2001; Genestra 2007; Gustafsson & Gottlieb 2009).

**mtDNA replication**

In order to maintain sufficient levels of ATP within the cell, mitochondria must have proper transcription and translation of the proteins that make up the electron transport chain (Moghaddas et al. 2003; Taylor et al. 2003). Because the majority of proteins encoded by mtDNA are necessary for electron transport chain function, it is important for cells to contain adequate copies of functional mtDNA. During embryonic development, ATP is crucial for the segregation of nuclear material and cell division, and the level of ATP present in blastomeres is
associated with mtDNA abundance (Van Blerkom et al. 2000). Oocytes must contain certain levels of mtDNA in order to successfully reach implantation and continue future development (Wai et al. 2010). This is because although mtDNA replication is paused between metaphase II and blastocyst implantation, the embryo is rapidly dividing during this period (Pikó & Taylor 1987; Thundathil et al. 2005). Cellular division and chromosomal segregation are energy-intensive processes, so if the original oocyte lacks proper mtDNA copy numbers, the embryo will not have enough energy to continue development as mitochondria become increasingly segregated with each cell division (Wai et al. 2010).

Competent oocytes have greater numbers of mtDNA than incompetent oocytes, and impaired mtDNA replication during oocyte maturation arrests development around the 6-cell stage (Spikings et al. 2007). Additionally, the fertility outcomes of incompetent oocytes can be recovered by supplementing with mitochondria from competent oocytes (El Shourbagy et al. 2006). In one study, competence of individual porcine oocytes was evaluated by the presence or absence of glucose-6-phosphate dehydrogenase (G6PD) activity (Spikings et al. 2007). Incompetent oocytes have ongoing G6PD activity whereas competent oocytes lose this enzyme; therefore the activity of G6PD can be utilized through staining assays to determine overall oocyte competence (Rodríguez-González et al. 2002).

The influence of mtDNA replication on oocyte competence can be examined to determine the effects of decreased copy numbers on embryonic development (Spikings et al. 2007). In one study, oocytes were exposed 2', 3'-dideoxycytidine (ddC) during in vitro maturation to inhibit mtDNA replication. ddC disrupts replication by incorporating into newly formed mtDNA to cause termination of the nucleoside chain, and cannot be removed by polymerase gamma exonucleases (Spikings et al. 2007). Surprisingly, no difference in
fertilization ability was found between ddC-treated and untreated competent oocytes, but major differences occurred during further embryogenesis. Oocytes with mtDNA replication disrupted by ddC failed to develop beyond the 6-cell stage (Spikings et al. 2007). This shows that while mtDNA copy number may have little effect on fertilization, mtDNA replication during oocyte maturation is important to increase copy number to supply future embryonic development.

**mtDNA copy number threshold**

While it is understood that a certain number of mtDNA copies are important for proper embryonic development, it is crucial to understand the threshold that defines competent and incompetent oocytes. This may be especially important in older women seeking to become pregnant, as low mtDNA copy numbers in oocytes have been associated with increased maternal age (De Boer et al. 1999). Previous research has shown that low mtDNA copy numbers do not appear to impair fertilization, but do have negative effects on post-implantation development (Wai et al. 2010; Spikings et al. 2007). Although healthy, wild-type mouse oocytes have a range of 11,000 to 428,000 copies of mtDNA, only 9 out of 219 embryos examined in one study contained less than 50,000 (Wai et al. 2010). This shows that while the number of mtDNA copies within healthy oocytes varies considerably, a certain threshold for competence is most likely present.

To study the effects of low mtDNA copy number on oocyte fertilization and embryonic development, heterozygous *Tfam* knockout mice can be used to create mice with low levels of mtDNA (Wai et al. 2010). The TFAM (mitochondrial transcription factor A) protein is important for mtDNA transcription and mitochondrial chromatin packaging, and therefore has been suggested as a regulator of mtDNA copy number (Kaufman et al. 2007; Ekstrand et al.
2004). Genetically manipulated *Tfam* knockout mice were used to examine the effects of low oocyte mtDNA copy number on fertilization and embryonic development. Low-copy oocytes show normal fertilization and preimplantation development, but arrest shortly after the start of embryogenesis (Wai et al. 2010). Similar outcomes are observed when low-copy embryos are transplanted into wild-type pseudopregnant females, indicating that the impairment lies within the oocyte itself rather than the maternal host environment (Wai et al. 2010).

These experiments revealed an estimated requirement of at least 50,000 mtDNA copies for murine oocytes to successfully progress through development after blastocyst implantation (Wai et al. 2010); human metaphase II oocytes show a similar threshold, with reported healthy mtDNA copy numbers ranging from 50,000 to 1,500,000 (Reynier et al. 2001; P May-Panloup et al. 2005). While healthy mammalian oocytes contain a wide range of mtDNA copy numbers, a certain threshold exists, below which the mtDNA population cannot rebound from the successive rounds of cleavage necessary for embryonic development (Wai et al. 2010). Because mtDNA copy numbers have been shown to decrease with maternal age (De Boer et al. 1999), this may be why older women struggle with maintaining pregnancies beyond fertilization.

**mtDNA integrity**

For proper mitochondrial function, mtDNA levels must be adequate in both quantity and quality (Wai et al. 2010; Copeland & Longley 2014). Like nuclear DNA, mtDNA is vulnerable to mutations and damage in both somatic and germline cells, but lacks many of the protective mechanisms that help maintain nuclear DNA integrity, such as histones and non-coding regions (Parsons et al. 1997). Several DNA polymerases maintain the integrity of nuclear DNA, but it is thought that these mechanisms are inefficient in repairing prokaryote-like mtDNA (Bentov et al. 72x745)
Only DNA polymerase-\(\gamma\) (POL\(\gamma\)) is known to function in mitochondria to maintain mtDNA integrity (Bebenek & Kunkel 2004), so it is not surprising that mutations in \(Pol\gamma\) can cause significant mitochondrial disorders (reviewed in Chan & Copeland, 2009). Experiments with mutations in \(Pol\gamma\) show that loss of this polymerase leads to increased ROS production and overall mitochondrial dysfunction, often observed in aging (Lewis et al. 2007; Yen et al. 1989; Taylor et al. 2003).

**mtDNA mutations**

Natural aging causes mtDNA mutations in many somatic tissues, such as the brain, liver, skeletal and cardiac muscle (Cortopassi et al. 1992; Cortopassi & Arnheim 1990; Corral-Debrinski et al. 1992; Yen et al. 1989), as well as oocytes (Keefe et al. 1995). Even in tissues such as germline cells that may remain quiescent for decades, low levels of damaging ROS build up over time to cause gradual mtDNA damage (Parsons et al. 1997). When natural protective mechanisms against oxidative damage are overwhelmed by the increase in ROS production with age, ROS begin to cause damage to proteins, lipids, and nucleic acids (Herrero & Barja 1997; Moghaddas et al. 2003; Haigis & Yankner 2010). Although mtDNA mutations can cause severe cellular dysfunction, it is suggested that mutations must accumulate within 30-80% of the mtDNA population in order for a phenotype to be expressed (Lewis et al. 2007). Studies of mutations such as the common 4977 deletion have shown that mutations are more prevalent in oocytes from aged women compared to younger women (Keefe et al. 1995). Surprisingly, large-scale deletions such as mtDNA\(^{4977}\) are rarely passed on to offspring; the mechanisms of this transmission barrier are unknown, but a genetic bottleneck for mtDNA selection has been proposed (Jansen & Burton 2004; Shoubridge & Wai 2007; Chinnery et al. 2004; Steffann et al.
Although mtDNA selection is random, a genetic bottleneck may sufficiently limit the risk of large mutations passing onto offspring (Chan & Copeland 2009; Fan et al. 2008; Corral-Debrinski et al. 1992).

Mitochondrial membrane potential

While mtDNA integrity is necessary for the transcription and translation of important mitochondrial proteins involved in oxidative phosphorylation, the mitochondrial membrane potential must also be maintained in order to successfully produce ATP and carry out additional mitochondrial functions (Mitchell & Moyle 1967; Shigenaga et al. 1994; Voisine et al. 1999). A high mitochondrial membrane potential (m$\Delta$Ψ) across the inner mitochondrial membrane is required to create the proton gradient involved in ATP generation during oxidative phosphorylation (Wilding et al. 2003). When the m$\Delta$Ψ is disrupted or decreased with age, adequate amounts of ATP are no longer produced by mitochondria within the oocyte and several cellular functions begin to fail (Komatsu et al. 2014). A strong association exists in human embryos between low m$\Delta$Ψ and abnormal meiotic spindle apparatuses (Wilding et al. 2003). When data from these studies are separated by donor age, oocytes from women over the age of 37 years show dramatic increases in rates of spindle abnormalities and chaotic mosaicism, characterized by the random segregation of chromosomes during meiosis (Wilding et al. 2003). These data indicate that not only do the oocytes of older women show decreased m$\Delta$Ψ, but this low m$\Delta$Ψ with age also increases the chance of abnormalities during embryonic development.

Conclusions
As the main energy producers of the cell, mitochondria play a large role in the maintenance of cellular health and viability. Age-related studies regularly focus on mitochondrial function as a benchmark of cellular health as many diseases and cancers are associated with mitochondrial dysfunction and mtDNA damage (reviewed in López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013). Because oocytes contain more mitochondria than any other cell type (Jansen & de Boer 1998), it is important to understand how mitochondrial function and dysfunction affect oocyte quality. While it is understood that oocyte quality eventually decreases with age (Fragouli & Wells 2011; Hook 1981; Kim et al. 2013; Nybo et al. 2000; Yoon et al. 1996; Gaulden 1992; Schwarzer et al. 2014; Hassold & Chiu 1985), it is not clear the exact mechanism by which this happens. Advanced age leads to increased mitochondrial dysfunction, which, through multiple pathways, ultimately results in reduced levels of ATP (Michikawa 1999; Taylor et al. 2003; Jansen & Burton 2004; Müller-Höcker et al. 1996; Eichenlaub-Ritter et al. 2011; Yen et al. 1989). Without the ability to produce an adequate energy supply, oocytes are unable to carry out basic cell functions.
CHAPTER 4: Oocyte quality decreases with age

Because mitochondria are the primary energy source for the oocyte, it is not surprising that loss of mitochondrial function can have detrimental effects on oocyte quality (Takeo et al. 2013; Bentov et al. 2011; Ford 2013; Van Blerkom et al. 1995). When mitochondrial dysfunction occurs, sufficient levels of ATP are no longer provided to carry out the cellular functions of the oocyte and problems can arise in oogenesis and embryogenesis once an oocyte becomes fertilized (May-Panloup et al. 2007; Van Blerkom et al. 1995). These problems typically manifest as pre or post implantation developmental arrest, implantation failure, spontaneous abortion, or developmental defects in the offspring resulting from aneuploidy (Robinson et al. 2001; Gaulden 1992; Allen et al. 2009; Hassold & Chiu 1985; Kushnir et al. 2012; Keefe et al. 1995).

Many studies have observed the increased risk of aneuploidy that is often associated with increased maternal age (Fragouli & Wells 2011; Yoon et al. 1996; Hook 1981; Nybo Andersen et al. 2000; Kim et al. 2013). Aneuploidy is the term used to describe any cell or group of cells that contain an incorrect number of chromosomes, typically indicated by one too many (trisomic) or one too few (monosomic) in a given chromosome pair. While approximately 20% of all human oocytes contain an abnormal number of chromosomes, oocytes from older women show aberrations in up to 50% (Hassold et al. 2007; Fragouli et al. 2011).

Oocyte quality has been observed in many studies to decrease with age, prompting the acceptance of what is known as the “maternal age effect” (Schwarzer et al. 2014; Rowsey et al. 2013; Gaulden 1992; Hassold & Chiu 1985). Although it is widely accepted that maternal age is associated with a decline in oocyte quality, it is undecided as to what exactly contributes to this phenomenon in oocytes (Rowsey et al. 2013; Hultén et al. 2010). This chapter will discuss how
mitochondrial dysfunction manifests in the aging oocyte and how the resulting abnormalities affect birth outcomes, drawing from results and statistics generated from various published literature. It will also examine a few of the current theories on the cause (or causes) of the maternal age effect, and discuss briefly the rapid decline in oocyte quality observed in pathologies separate from maternal aging alone.

**Spindle assembly**

Perhaps the most important component of cell division is the spindle. Made up of microtubules composed of α and β tubulin dimers, the spindle is highly ATP-dependent and is responsible for the segregation of chromosomes during cell division. Unlike the spindle apparatus of somatic cells, oocytes lack centrosomes (reviewed in Dumont & Desai, 2012). Instead of microtubules nucleating from centrioles within centrosomes, oocytes contain microtubule-organizing centers (MTOCs) adjacent to chromosomes within the cell and create an “inside-out” formation of the spindle poles (reviewed in Chapter 1).

Studies have focused on the mechanics of the spindle apparatus itself and how its dynamics can be altered by maternal aging (Battaglia et al. 1996; Vogt et al. 2008; Howe & FitzHarris 2013). In one study, Battaglia *et al.* (1996) examined metaphase II oocytes from two populations of women: 40 to 45 years old (aged) and 20 to 25 years old (young). Using high-resolution confocal microscopy to visualize fluorescently labeled chromatin and α-tubulin, it was discovered that oocytes from aged women displayed increased spindle disruptions and abnormal chromosome segregation compared to oocytes from younger women. Conditions consistent with aneuploidy were observed in 79% of aged oocytes, but only 17% of young oocytes displayed the same phenotypes (Battaglia et al. 1996). These results indicate that the increase of spindle
abnormalities with age likely contributes to observed increases in aneuploidy, and it is suggested that mitochondrial dysfunction contributes these disruptions in spindle function (Battaglia et al. 1996; Howe & FitzHarris 2013; Vogt et al. 2008).

**Spindle assembly checkpoint**

In most dividing cells, certain checkpoints are in place to ensure that aneuploidy does not occur. One such checkpoint is the spindle assembly checkpoint (SAC) that prevents cells from entering anaphase without proper spindle-chromosomal attachment (Musacchio & Salmon 2007; Musacchio 2011). This signal must be silenced in order for anaphase to begin. In mitosis, proteins of the mitotic checkpoint complex (MCC) are produced to bind to and block activation of the anaphase promoting factor/cyclosome (APC/C) (Musacchio & Salmon 2007; Howe & FitzHarris 2013). When correct spindle attachments are detected, the cell no longer produces MCC, leaving the APC/C available to activate anaphase (Musacchio & Salmon 2007).

Meiosis in male gametes uses a similar mechanism to prevent aneuploidy in developing sperm. When the correct spindle attachments are not present, the cell cycle is arrested and cell death occurs (Musacchio 2011). However, this checkpoint in female gametes appears to be much less stringent, allowing for aneuploidy to occur without delay of the cell cycle (LeMaire-Adkins et al. 1997). This may be the reason why the occurrence of aneuploidy is much greater in oogenesis than spermatogenesis (Pacchierotti et al. 2007). It has been shown that anaphase can still proceed in oocytes where one or more chromosomes is incorrectly attached to the spindle (Kouznetsova et al. 2007; Nagaoka et al. 2011), leaving the SAC extremely prone to errors during oogenesis and further development (Gui & Homer 2012; Kolano et al. 2012). Therefore, it is no surprise that as mitochondrial function declines with advanced maternal age, oocytes
lacking proper spindle assembly and/or the energy to make proper microtubule-chromosome attachments have a greater chance of bypassing cell cycle checkpoints than other cell types.

**Premature separation of sister chromatids and recombination failure**

Aneuploidy can occur as the result of a variety of events, such as recombination failure, lack of segregation of homologous chromosomes or sister chromatids (nondisjunction), and premature separation of sister chromatids (PSSC) (FIGURE 2) (reviewed in So I Nagaoka et al., 2012). Based on data from incidences of aneuploidy in spontaneous abortions, stillbirths, and live births, it has been estimated that approximately 5% of all fertilized oocytes are aneuploid (Hassold & Hunt 2001), although not all survive until birth. Oocytes must go through two rounds of meiosis from the primordial germ cell stage to fertilization, which involves two cell divisions without DNA replication (as seen in mitosis). As a result, two polar bodies are formed – one during the first meiotic division and the other during the second (FIGURE 3). The correct number of chromosomes must be segregated into each so that the oocyte contains the appropriate amount of genetic material when combined with a sperm cell.

When chromosomes or chromatid pairs do not separate at the appropriate time during cell division, it can cause too many or too few chromosomes within the developing embryo, leading to aneuploidy. The extent of aneuploidy depends on the time and location at which the error takes place. Errors in meiosis I can be caused by recombination failure, premature separation of sister chromatids, and nondisjunction, whereas nondisjunction and PSSC cause errors in meiosis II (FIGURE 2); however, trisomies are more commonly caused by errors in meiosis I (Gaulden 1992).
Many aneuploidies are caused by the failure of homologous chromosomes to recombine or create successful “crossovers” between chromosomes during the early stages of meiosis in the fetal ovary (Hassold & Hunt 2001). In order for meiotic recombination to be successful, chromosome pairs must contain crossovers that allow for the exchange of genetic information between the two chromosomes (Smith & Nicolas 1998). Joined pairs of homologous chromosomes, known as bivalents, that lack crossovers are expected to result in aneuploidy 50% of the time (Cheng et al. 2009). Studies have shown that more than 10% of all oocytes possess one of these “crossover-less” bivalents, yet almost all bivalents in males have at least one crossover joining the two homologous chromosomes (Cheng et al. 2009; Lynn et al. 2002).

**Spontaneous abortion and miscarriage**

Developmental arrest, failure to implant into the uterine wall, and spontaneous abortions can all be induced by aneuploidy (Fragouli & Wells 2011). Each of these lead to miscarriage, the incidence of which typically increases with advanced maternal age (Nybo et al. 2000). Most spontaneous abortions (characterized by fetal death occurring between 6 and 20 weeks gestation) are caused by random segregation errors during meiosis, and the frequency of these errors increases as a woman ages (Robinson et al. 2001). The chromosomal abnormality attributed to a woman’s first miscarriage does not influence the chance of the woman having an additional spontaneous abortion with the same abnormality (Robinson et al. 2001). Therefore, the likelihood of a woman having a certain abnormality is not dependent on which abnormalities caused her previous spontaneous abortions, indicating that the segregation errors observed are indeed random. Based on these results, scientists have concluded that maternal age is the main
factor involved in predicting which cohorts of women will experience more of these random segregation errors that lead to spontaneous abortion (Robinson et al. 2001).

Additional studies have discovered that a woman in her early 20’s has approximately a 9% risk for miscarriage, and this risk increases to over 75% for a woman over 45 years of age (Nybo et al. 2000). It is not surprising, therefore, that 2-3% of embryos from women in their 20’s carry trisomies, while 35% of embryos from women in their 40’s are trisomic (Hassold & Chiu 1985). Embryos with trisomies in some chromosomal pairs are able to survive, but overall, trisomic embryos are 3 times less likely to develop to the blastocyst stage than euploid embryos (Sher et al. 2007). A study of clinically diagnosed miscarriages of women older and younger than 35 years of age found that chromosomal abnormalities were present in 50% of the miscarriages of younger women and 75% of the miscarriages of older women (Nybo Andersen et al. 2000). These data show that while increased chromosomal abnormalities play a role in the risk for aneuploidy, additional factors may also contribute to miscarriage. It has been suggested that chromosomal abnormalities may make certain oocytes more susceptible to future insults by additional environmental factors that can cause mtDNA damage or loss of regulatory hormones (Hassold & Sherman 2000).

**Trisomy 21 risks**

While aneuploidy can occur in any of the 23 pairs of chromosomes, many aneuploid embryos are arrested during development and do not survive to birth (Hassold & Hunt 2001). Live births have been reported in cases of certain trisomies, such as 21 and sex-chromosomal trisomies, although the risk for each depends on the chromosome (Risch et al. 1986). For example, different trisomies display different correlations with maternal age, although the overall
trend for trisomies shows an increased risk with advanced age (Kim et al. 2013). The origin of the trisomy also depends on the individual chromosome, with some deriving from meiosis I, and others from meiosis II (reviewed in Nagaoka et al. 2012). Trisomies that occur in meiosis I are typically a result of nondisjunction of the homologous chromosomes, recombination failure, or premature separation of sister chromatids, whereas trisomies in meiosis II are thought to be caused by nondisjunction of the sister chromatids (Hassold & Hunt 2001).

Trisomy 21, typically known as Down syndrome, is one of the more commonly observed trisomies since it can arise from 3 different recombination error scenarios (reviewed in Nagaoka et al. 2012) (FIGURE 4) (versus one scenario in other trisomies), and more than 5,000 children born each year in the United States are affected (Canfield et al. 2006). Like many other trisomies, the risk of Down syndrome increases with advanced maternal age (Kim et al. 2013). While the risk of a woman aged 20 to 24 years giving birth to a child with Down syndrome is approximately 1 in 1400, this risk increases exponentially with age. At 35 years old risk increases to 1 in 350, at 40 years old risk increases to 1 in 60, at 45 years old risk increases to 1 in 25, and at 49 years old, this risk becomes 1 in 11 (Hook 1981; Yoon et al. 1996).

As mtDNA is inherited maternally from mother to daughter, it is easily understood how this risk for trisomy 21 also appears to increase with an advanced grand-maternal age at the time of the mother’s birth (Aagesen et al. 1984; Malini & Ramachandra 2006). Studies of this increased risk found that women had a 30% increased risk for giving birth to a child with Down syndrome for every year their own mother’s age exceeded 30 years old at the time of their birth (Aagesen et al. 1984; Malini & Ramachandra 2006). These studies suggest that while it is possible for women of advanced maternal age to give birth to healthy children, it may increase their daughters’ chances of having oocytes with increased levels of chromosomal abnormalities.
Limitations to diagnosis and research

Although there are a vast number of studies on aneuploidy and chromosomal abnormalities, results must be approached with a certain degree of caution. As many studies on oocyte quality in humans are performed on individuals seeking fertility treatments, it is unclear whether these women are merely more likely to harbor oocyte abnormalities and other reasons for reduced infertility, or if their results do in fact represent human females as a whole. Factors causing chromosomal abnormalities or increased risk of aneuploidy with age may simply be restricted to sub fertile or infertile advanced-age women, or this group may be more susceptible to influence by hormonal or regulatory factors later in life. Interestingly enough, it has also been suggested that predictions or risks associated with age may be more closely connected to biological age rather than chronological age (Kline et al. 2000). In a study of post-menopausal women who had previously experienced spontaneous abortions in their lifetimes, it was discovered that women with karyotyped trisomic aborted embryos typically entered menopause 1 year earlier than those women with chromosomally normal aborted embryos (Kline et al. 2000). Either way, women and couples seeking assisted reproductive technologies do not necessarily represent the general population in many studies. One exception to this commonality is the study of spindle abnormalities by Battaglia et al. (1996). This study used oocytes from naturally cycling women with no history of infertility or poor health; therefore, researchers were able to remove any bias that may be introduced from cohorts of women seeking infertility treatments and still observed dramatic differences with age (Battaglia et al. 1996).

An additional limitation to trisomy research is that many aneuploidy studies are performed retrospectively, meaning that the trisomic child has already been born. This limits
scientists’ access to only a certain type of available subjects, and not every case of trisomy that may have caused implantation failure or spontaneous abortion. Rather than examine aneuploidy retrospectively, more information about the chromosomal abnormality is often gained when oocytes are examined before implantation. This is often accomplished either by taking a biopsy of a single blastomere of the early embryo, or analyzing the polar bodies produced during meiosis (reviewed in Nagaoka et al. 2012). Although this is typically only performed as a technique in assisted reproduction therapies when choosing embryos for transfer, it is perhaps the most reliable method to date for determining the genetic profile of an embryo.

Despite caveats involved in this type of research, it must be noted that there are in fact observable differences between oocytes from young and aged women, even if these women are from a specific subset of the entire population. Though analysis techniques and restricted variability in sample size may exaggerate the presence of certain aneuploidies, it is clear that intrinsic differences are present before scientific intervention for such disparities to occur between experimentalss and controls.

**Long meiotic arrest results in mtDNA damage**

These differences between young and aged oocytes have often been attributed to what is known as the maternal age effect. Trisomies, miscarriage, implantation failure, and developmental arrest have all been observed to increase with maternal age (Vialard et al. 2007; Gaulden 1992; Allen et al. 2009; Hassold & Chiu 1985; Kim et al. 2013; Robinson et al. 2001). Some studies point to a single cause, while others adopt a multi-hit mechanism (reviewed in Rowsey et al. 2013). While it is unclear what exactly causes this maternal age effect, it is generally speculated that it is not the result of a single factor, but may be the work of several in
combination (reviewed in Rowsey et al. 2013). Many theories point to possible mechanisms by which the maternal age effect acts to decrease oocyte quality, each of which most likely contribute to the overall effect. One of these theories focuses on the amount of time oocytes spend arrested in prophase during adult life (Wang & Höög 2006). Oocytes enter meiosis I during fetal development and arrest in late prophase until the LH surge during ovulation stimulates the continuation and completion of meiosis I. Because some women delay childbirth until later in reproductive life, oocytes generated during fetal development may be exposed to ROS that cause mtDNA damage for decades before ovulation (Wang & Höög 2006). Although oocyte mitochondria are relatively inactive compared to somatic mitochondria, low levels of ATP and ROS are still produced, and mtDNA damage can accumulate over time (Amicarelli et al. 2003; Wang et al. 2013; Dai et al. 2014). Mitochondrial dysfunction in oocytes is often associated with an increased incidence of longer time to fertilization, implantation failure, and developmental arrest (Van Blerkom et al. 1995; Van Blerkom et al. 2000; Keefe et al. 1995). It may be possible that with age, oocytes that have been exposed to more mtDNA damage may be more likely to become aneuploid when meiosis resumes.

**Cohesion loss**

Another promising theory for the maternal age effect is the loss of certain proteins over time that contributes to proper chromosomal segregation during meiosis. For instance, the cohesion complex is made up of specific proteins that hold the chromatids together during prophase (FIGURE 5). Cohesion proteins are responsible for securing the homologous chromosomes as well as each sister chromosome to its pair to keep them together until the cell is ready to segregate each into the dividing cell (reviewed in Hunt & Hassold 2010). In
homologous chromosomes, the chromatin complex is present around the arms of each to keep the pairs together; in sister chromatids it is found around the kinetochores (Nasmyth 2011). When the cell is signaled to enter into anaphase and segregate the chromosomes, cohesion is released from the appropriate region of the pair (either the arms or the kinetochores), allowing the chromosomes to segregate to their designated cellular poles. If cohesion is prematurely lost or degraded, the chromosome pairs fail to join tightly enough to keep them together during various cell cycle stages. This can cause PSSC, which has been shown to play a major role in the occurrence of aneuploidy (Angell 1991).

In both *Drosophila melanogaster* and mice, loss of cohesion proteins results in increased rates of aneuploidy (Jeffreys et al. 2003; Hodges et al. 2005) and this phenomenon has also been observed in humans (Tsutsumi et al. 2014). In a recent study by Tsutsumi *et al.*, fluorescence microscopy was used to identify levels of two cohesion complex subunits specific to meiosis in human oocytes, REC8 and SMC1β (2014). The study examined oocytes arrested in prophase I from samples of ovarian tissue of women aged 19 years to 49 years divided into “29 and below” and “40 and above” cohorts. Comparisons showed an overall decline with increased age in the average intensity levels compared to the internal control (Tsutsumi et al. 2014). Because certain cohesion proteins are produced during the S-phase of the cell cycle in the fetal ovary, it is not surprising that with increased maternal age, levels of these proteins within oocytes generated during fetal development decrease (Tsutsumi et al. 2014). In a related study, mice with mutated *Smc1b* demonstrated increased levels of aneuploidy (Hodges et al. 2005), suggesting that aneuploidy is caused by the loss of these cohesion proteins. To further support these findings, naturally-aged mice show an increased distance between sister chromatid kinetochores when compared to younger mice at metaphase, indicating a natural loss of cohesion proteins with age.
(Chiang et al. 2010). Together, these studies show that the loss of cohesion contributes to the maternal age effect in oocytes, and that the resulting increase in aneuploid embryos may be a result of the lost ability for the cell to select against trisomic oocytes.

**Carbonyl stress**

In addition to increases in reactive oxygen species, the maternal age effect has also been attributed to an increase in reactive carbonyl species (RCS). These molecules are products of metabolic glycolysis and have many of the same functions as ROS (Mano 2012). However, these carbonyls are more stable than ROS, which allows them to travel greater distances in the cell and to have a more damaging effect. Through glycation of proteins, RCSs cause post-translational modifications and impair protein function in the cell (Mano 2012). To counteract damage generated by natural metabolism, cells contain glyoxylases such as GLO1 to inactivate RCSs and prevent the formation of advanced glycation end-products (AGEs) (Thornalley, 2003; Dobler, Ahmed, Song, Eboigbodin, & Thornalley, 2006). The most destructive RCS is methylglyoxal (MG) that is derived from carbohydrate metabolism and can have detrimental effects on mitochondrial function (Amicarelli et al. 2003; Thornalley 2008). Reactive carbonyl species can also create a positive feedback loop with ROS production to further cellular damage; ROS promote the final step of protein glycation by RCS, and AGEs promote pathways that induce ROS formation (Tatone & Amicarelli 2013). The first study to link RCS and ovarian dysfunction discovered that women with polycystic ovarian syndrome (PCOS) had higher levels of AGEs than control women of the same age (Diamanti-Kandarakis et al. 2007). It was also discovered in mice that the ovaries of older females display increased levels of AGEs and decreased activity of GLO1, indicating that cells are no longer able to protect against AGE-
induced damage (Tatone et al. 2010). While these studies focused on the ovarian environment rather than oocytes specifically, the data show that the microenvironment of the oocyte within the aging ovary may also play a large role in maintaining quality throughout adult life.

**Oogonial stem cells**

While many of the previously mentioned studies point to accumulation of damage to arrested oocytes during adult life, there is still the question of how aging affects germline stem cells and how these contribute to the aging phenotype. Although research in the last decade has shown that oogonial stem cells (OSCs) exist in mammals (Johnson et al. 2004; Zou et al. 2009; White et al. 2012), many wonder why menopause occurs if not for the exhaustion of a fixed follicular pool. It has been suggested that women undergo menopause due to the lack of necessity to have children past the fifth and sixth decade of life (Cohen 2004), and/or to limit the time of reproduction so that cellular function can be directed towards maintaining the health of the mother later in life rather than allocating resources for the production of offspring (Kirkwood 2005). Through transplantation studies (Niikura et al. 2009) it has been demonstrated that the health of the host ovarian niche is extremely important in predicting the success of oocyte development and competence. Though ovarian tissue from older mice can successfully produce immature follicles when transplanted into younger ovaries, the reverse procedure often fails (Niikura et al. 2009). This indicates that while oogonial stem cells may continually contribute to the follicular pool during post-fetal life, the aging ovarian environment may reduce the ability of OSCs to produce viable follicles.

**Diabetes and obesity affect oocyte quality**
Although many couples and individuals seek fertility assistance due to advanced maternal age, there are also other pathologies that affect oocyte quality in women of all ages. While maternal aging is a heavily researched topic in the field of female reproduction, the effects of maternal obesity and diabetes on oocyte quality have also been the focus of many studies (Wang & Moley 2010; Diamond et al. 1989; Wang et al. 2009; Moley et al. 1991; Grindler & Moley 2013; Purcell & Moley 2011). Oocytes from aged women and diabetic women often display similar phenotypes, indicating that technologies and therapies to improve oocyte quality may be able to benefit both demographics. Mitochondrial structural abnormalities, mitochondrial aggregation, decreased mitochondrial function, increased incidence of miscarriage, spindle defects, and implantation failure are all characteristics of both aged and diabetic oocytes (Simsek-Duran et al. 2013; Komatsu et al. 2014; Wilding et al. 2003; Robinson et al. 2001; Gaulden 1992; Wang et al. 2009; Colton et al. 2002; Tarín et al. 2001). Metaphase II oocytes from diabetic mice also show increased levels of aneuploidy (Wang et al. 2009) similar to increases in aneuploidy with maternal age (Hassold & Hunt 2001; Fragouli & Wells 2011).

In addition to mitochondrial dysfunction, maternal diabetes can disrupt important cell-to-cell communication between granulosa cells and the oocyte (Ratchford et al. 2008). Diabetic oocytes transferred into non-diabetic host uterine environments as early as the one-cell stage still display cellular defects (Diamond et al. 1989; Moley et al. 1991; Wyman et al. 2008), suggesting that the negative effects attributed to maternal diabetes are able to influence oocyte quality in the early stages of oogenesis and embryogenesis. At the blastocyst stage, embryos from diabetic mothers show reduced levels of glucose transport with down-regulated expression of glucose transport proteins (Moley 1999) which is consistent with increased levels of apoptosis (Moley et al. 1998).
Obesity is another condition that affects oocyte quality and can become a fertility issue for women of all ages. Almost one third of all women of reproductive age in the United States are currently considered to have a body mass index (BMI) in the “obese” range of 30 and above (Hillemeier et al. 2011; Flegal et al. 2010). While the causes for reduced fertility in obese women is currently debated (reviewed in Purcell & Moley, 2011), it is clear that oocyte quality is affected. Obese women are approximately three times less likely to conceive than normal weight women, even when cycling normally, and display increased risks of miscarriage and birth defects when conception occurs (Jungheim & Moley 2010; Rich-Edwards et al. 1994). Obese women are also more likely to develop PCOS, which has further detrimental effects on fertility (Norman et al. 2007).

Conclusions

Based on studies of maternal aging, diabetes, and obesity, it is clear that decreases in oocyte quality can arise from a number of factors (Wang & Moley 2010; Purcell & Moley 2011; Korkmaz et al. 2014). Treatments to improve oocyte quality in women of advanced age may also contribute to helping women with diabetes or obesity overcome the subfertility or infertility associated with these conditions. While the maternal age effect on oocyte quality is quite established, it is still debated as to which factors play the largest role in determining oocyte competence in advanced maternal age. It is possible that abnormalities during early oocyte development make certain women more susceptible to subfertility or infertility during adult life, or perhaps the maternal age effect is due to a breakdown of certain quality control mechanisms present in younger ovaries (reviewed in Rowsey et al. 2013; reviewed in Nagaoka et al. 2012). The deterioration of the ovarian microenvironment may also contribute to poor oocyte quality in
older women. Until these details are better understood, assisted reproductive treatments cannot be completely optimized, and though risk factors may vary between individuals, this area of study could certainly benefit from additional clarification on female human reproduction as a whole.
CHAPTER 5: Strategies for improving oocyte quality

As more and more women delay childbirth until later in life, the need for successful assisted reproductive technologies becomes more apparent. Birth rates in women in their 30’s and early 40’s in 2012 increased from rates in 2011, while rates for every other age group dropped, showing an increase in the number of women waiting to have children (Hamilton et al. 2013). In fact, approximately 20% of women now give birth to their first child after the age of 35, although one-third of these women experience some form of subfertility problem (CDC 2013). According to the Center for Disease Control 2011 ART Clinical Report, approximately 11-12% of all reproductive-age women in the United States have difficulty becoming pregnant or maintaining pregnancy naturally and have sought out some sort of fertility treatment or testing in their lifetimes (CDC 2013). While the number of women receiving fertility assistance is growing, pregnancy success still often depends on the age of the female. Women less than 35 years of age using fertility treatments with their own oocytes typically experience a success rate of 40%, but this number quickly declines to 22% at age 40 and 1% at age 44 (CDC 2013). Women of advanced age seeking infertility assistance face increased risks of miscarriage, aneuploidy, and implantation failure compared to younger women (te Velde & Pearson 2002). Although many current fertility treatments have been successful, none have been able to reach 100% rates for all women. Because reasons for and degree of infertility can vary between women, researchers and clinicians must be able to provide individual resources depending on what is needed to assist reproduction (te Velde & Pearson 2002). Until research can advance enough to produce individual treatments, current therapies must be improved to increase oocyte quality and embryonic development in both women of advanced age and women with premature
ovarian failure. This chapter will discuss the methods used in typical assisted reproductive treatments and highlight recent progress in the field to suggest possible future directions.

**How is infertility/subfertility treated?**

Assisted reproductive technologies typically first begin with hormone treatments to promote follicle growth and ovulation. It is debated over whether or not these exogenous hormones have adverse effects on oocyte quality, as many studies have observed increased in aneuploidy and epigenetic changes in oocytes exposed to high doses (Doherty et al. 2000; Khosla et al. 2001; Rivera et al. 2008; Market-Velker et al. 2010; Munne et al. 1997). However, more recent methods have begun to see improvements in embryo quality with lower doses of hormones, suggesting that the regulation and maintenance of the oocyte environment is highly sensitive to change (Baart et al. 2007; Rubio et al. 2010). Once oocytes are collected, they are fertilized by either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (FIGURE 6). The first procedure involves mixing oocytes with sperm cells in a dish, while the latter injects sperm directly into an oocyte (reviewed in Nagaoka et al. 2012). After fertilization, the zygotes are then cultured until further use.

**Preimplantation genetic diagnosis**

After early stage embryos are collected, they are often screened for aneuploidy (Munné et al. 2010). This ensures that the best blastocysts with the greatest degree of potential success are transplanted into the mother. One of the polar bodies or blastomeres from the early blastocyst is used for genomic analysis before implantation. For many years, fluorescence in situ hybridization (FISH) has been used as the primary means for karyotyping trisomies within a
given blastocyst (Hultén et al. 2010). However, this method has many limitations, the first of which being that many FISH analyses can only monitor a few chromosomes at a given time (reviewed in Rowsey et al. 2013). While this is helpful when determining rates of specific trisomies, additional aneuploidies in other chromosomes within the same cell that can have negative effects may go unnoticed. Therefore, the use of FISH for genetic analysis has been highly debated; many believe using FISH for preimplantation genetic diagnosis provides no benefit (Genetics 2009), while other scientists report improvements in pregnancy outcomes (Munné et al. 2010). These opposing results may depend on whether the aneuploidy could be detected from the limited number of probes chosen since all 23 chromosomes cannot be monitored with this method.

A more reliable method is comparative genomic hybridization (CGH) (reviewed in Nagaoka, Hassold, & Hunt, 2012), which can detect aneuploidy in all 23 chromosomes of a single cell. This method involves the amplification of experimental DNA, which is then compared to normal reference DNA using various fluorochromes to identify specific areas on each chromosome. The ratio of each is calculated and compared to determine any genetic defects present in the experimental DNA and examines all 23 pairs of chromosomes rather than a small subset in FISH protocols. Pairing this method with a microchip array (aCGH) containing each fluorochrome probe decreases the length of time it takes to receive results, which can be critical in the timing of in vitro fertilizations. While these methods of preimplantation genetic screening can be beneficial for some, they typically do not show any improvements in pregnancy success in women younger than 40 years old. However, for women over 40, preimplantation genetic screening can improve outcomes by twice as much (Milán et al. 2010). This information
reinforces the idea that fertility treatments must be tailored to individual women and not every treatment is ideal for the entire population.

Once the embryos with the highest chance of success have been chosen, they are either transferred into the mother or cryo-frozen for later use. While freezing embryos may appear to be a tempting procedure for young women to take preventative measures against decreased fertility later in life, it is actually more cost-efficient to forgo these procedures and deal with subfertility as it arises. A study of healthy women found that the cost of freezing embryos or ovarian tissue at age 25 was more expensive than using assisted reproductive treatments at age 40 (Hirshfeld-Cytron et al. 2012). In addition, some women did not experience difficulties with fertility at age 40 at all. These data further reinforce the idea that chronological age may not directly predict biological age, and that there is much variability between individuals of the same population.

**How can oocyte quality be improved?**

**Ooplasmic transfer**

While improving methods used in assisted reproductive treatments is extremely important, it cannot be forgotten that a major underlying factor is oocyte quality. If oocyte quality can be improved, it would reduce the need for improved ART protocols and may also decrease the number of women reliant on them. When scientists discovered that older patients could carry successful pregnancies if the oocyte was from a younger donor, it was determined that the oocyte is the major driving factor in pregnancy success (Sauer et al. 1995). This led scientists to believe that some component within young oocytes was lacking in older oocytes. To improve older oocytes, cytoplasm from young oocytes was injected during IVF procedures in
attempts to improve oocyte quality (FIGURE 7). Successful live births were derived from these experiments (Cohen et al. 1998), but the health of the resulting offspring was questioned when it was discovered that they had mitochondrial heteroplasmy (Barritt et al. 2001; Brenner et al. 2000). Because mitochondria are inherited maternally, these children contained mitochondria from both their mother and the woman who donated the young cytoplasm. The US Food and Drug Administration put a moratorium on “oooplasmic transfer” in 2001 due to the suspicion that this procedure involved genetic manipulation that had unknown results (Frankel & Chapman 2001; Parens & Juengst 2001). Mouse studies performed after this procedure was performed in humans found that mitochondrial heteroplasmy caused reduced mitochondrial function in adult offspring (Acton et al. 2007). While these procedures are no longer performed, they made it clear that the mitochondria of the young oocyte were the factor that improved quality of older oocytes, and focus began to shift towards improving mitochondria to maintain fertility later in life.

**AUGMENT – autologous germline mitochondrial energy transfer**

To improve mitochondrial quality in the aged oocyte while circumventing mitochondrial heteroplasmy, an autologous source of healthy mitochondria was needed. This became possible with the discovery and isolation of oogonial stem cells within female mammals, including humans (Johnson et al. 2004; White et al. 2012; Zou et al. 2009). OSCs allowed for the development of autologous germline mitochondrial energy transfer (AUGMENT), which involves the transfer of oogonial stem cell mitochondria to the oocyte of the same patient to rejuvenate the energy supply for the dividing embryo (FIGURE 8). Not only does AUGMENT provide an autologous supply of mitochondria to older oocytes, but it also supplies mitochondria
from the same developmental cell line. Since mitochondria from different tissues differ in function and protein expression (Johnson et al. 2007; Riva et al. 2005), it is important to use mitochondria that will respond to the correct developmental cues present within the oocyte microenvironment. This is one reason why, for instance, mitochondria from other somatic tissues are inefficient for AUGMENT (Takeda et al. 2005). Another reason why autologous somatic cell mitochondria should not be transferred into oocytes is because of accumulated mtDNA damage (Barja 2002; Copeland & Longley 2014; Corral-Debrinski et al. 1992). Just as mtDNA damage accumulates within oocyte mitochondria, it also affects somatic mtDNA with age. Therefore, oogonial stem cell mitochondria would provide the “purest” and most developmentally competent supply of mitochondria to poor quality oocytes with dysfunctional mitochondria. One caveat to this method is that the oogonial stem cells must be harvested from the patient and the mitochondria must be carefully isolated for the transfer of mitochondria to occur. Because these cells are found in the ovarian tissue, the process may be invasive and difficult to perform. Therefore, many treatments have turned to improving existing mitochondria rather than transferring healthy organelles into oocytes.

**Antioxidants**

Because ROS are considered the main culprit of mtDNA damage with age, many studies have focused on the use of antioxidants as preventative treatments for maternal age effects on oocyte quality. Reactive oxygen species are a natural byproduct of cellular metabolism and are most often produced at Complex I and Complex III in the electron transport chain of mitochondria (Lenaz 2012; Dröse & Brandt 2012). They play a role in many cell-signaling pathways such as the MAPK pathway and the PI3K pathway, and are also involved in
maintaining the immune response (Devadas et al. 2002; Genestra 2007; Tobiume et al. 2001; S.-R. Lee et al. 2002; Kwon et al. 2004; Seo et al. 2005). In order to keep high levels of ROS at bay, cells produce antioxidants that counter ROS production and damage to DNA and proteins (Valko et al. 2006; Chatterjee et al. 2007). These antioxidants include enzymatic and non-enzymatic varieties, the latter of which is divided into metabolic and nutrient types. The main enzymatic antioxidant is superoxide dismutase (SOD) that converts superoxide radicals into $\text{H}_2\text{O}_2$, which is then converted into $\text{H}_2\text{O}$ and $\text{O}_2$ by additional enzymatic antioxidants (Halliwell 2007). Non-enzymatic metabolic antioxidants include endogenous CoQ10, while exogenous antioxidants are derived from nutrients such as vitamin E, C, and omega-3 fatty acids (Willcox et al. 2004).

Although antioxidants appear to be a clear choice for maternal aging treatments, many studies focused on increasing endogenous and exogenous antioxidants conflict as to whether or not antioxidant treatments improve cellular longevity (reviewed in Dai, Chiao, Marcinek, Szeto, & Rabinovitch, 2014). One study demonstrated that the overexpression of SOD in transgenic mice surprisingly did not increase lifespan compared to wild type controls (Pérez et al. 2009). While some studies show benefits to antioxidants in the treatment of certain pathologies, others do not, implying that antioxidants may play different roles in the prevention or repair of different disease progressions (Myung et al. 2013; Li et al. 2012).

In regards to female reproduction, antioxidants do not appear to have practical applications in improving oocyte quality with increased maternal age. Two experiments in which the diets of female mice were supplemented with the same dosages of vitamins C and E showed varying benefits to antioxidant treatments (Juan J Tarín et al. 2002; J J Tarín et al. 2002). While one study discovered that the oocytes of antioxidant-treated aged mice showed normal
chromosomal distribution in comparison to younger mice (Juan J Tarín et al. 2002), the other demonstrated an extreme reduction in litter size and the survival of offspring (J J Tarín et al. 2002). These results show that while antioxidants may improve oocyte quality in aged females at first glance, they may have a detrimental effect on the uterine environment and/or embryonic development in the womb.

**Coenzyme Q10**

Coenzyme Q10 (CoQ10) is considered a non-enzymatic metabolic antioxidant that is naturally found in the electron transport chain of mitochondria. It functions to transport electrons from Complexes I and II to Complex III, and also transfers protons across the inner mitochondria membrane to increase ATP production (Rosenfeldt et al. 2003; Pignatti et al. 1980; Sandhir et al. 2014). As humans age, levels of CoQ10 decrease (Pignatti et al. 1980), so many treatments to counteract mitochondrial dysfunction with age have focused on CoQ10 dietary supplements. CoQ10 has shown improvements in many studies related to various mitochondrial disorders such as hypertension, Parkinson’s disease, and macular degeneration, and has also been effective in increasing mitochondrial activity in aged oocytes from mice (Bentov et al. 2010; Feher et al. 2005; Winkler-Stuck et al. 2004; Rosenfeldt et al. 2003). CoQ10 supplements have also shown increases in oocyte quality and menopausal cognitive effects in mice by affecting mitochondrial dysfunction and ROS production (Gendelman & Roth 2012; Sandhir et al. 2014). While these studies have yet to be performed on humans, preliminary work in bovine and mouse models has produced a promising outlook for CoQ10 treatments.

**Resveratrol**
The polyphenol resveratrol has also been a focus of increasing mitochondrial function and oocyte quality by acting as a potent antioxidant. Resveratrol is an activator of SIRT1, which has a key role in many mitochondrial functions including mitophagy and biogenesis to reduce oxidative stress levels (Gustafsson & Gottlieb 2009; Kang & Hwang 2009; Cantó et al. 2012). The inhibition of SIRT1 has been shown to cause decreased fertility in mice (Takeo et al. 2013). Although grapes, soybeans, and peanuts are all excellent sources of resveratrol, it is most commonly known as the antioxidant found in red wine (Burns et al. 2002). While high levels of alcohol consumption may counteract the benefits of resveratrol in red wine (Katsiki et al. 2014), data generated from studies using the isolated polyphenol in improving oocyte quality have shown promising results. One study examined oocytes cultured in vitro with resveratrol and monitored SIRT1 protein expression, mitochondrial function, and fertility rates during IVF (Takeo et al. 2014). Compared to controls, resveratrol increased SIRT1 expression, ATP content, mtDNA copy number, and mitochondrial membrane potential in in vitro matured oocytes, and also increased fertilization rates during IVF treatments (Takeo et al. 2014). These data not only show the antioxidant capacity of resveratrol to improve mitochondrial health, but also the ability to improve fertilization and quality of oocytes.

Recent resveratrol research also points to the possibility of providing improvements in mitochondrial function with age to increase the quality of oocytes. A study in mice compared oocyte quality and quantity in mice supplemented with resveratrol for 6-12 months to age-matched controls as well as young mice (Liu et al. 2013). Analysis after the 12-month period showed an increase in the follicular pool and overall number of oocytes in mice treated with resveratrol compared to aged mice without resveratrol. Oocyte quality, based on the alignment of chromosomes on the metaphase plate and proper spindle formation, was also improved.
compared to controls (Liu et al. 2013). This resulted in great differences in reproductive potential, as mice treated with resveratrol were able to maintain successful reproduction while age-matched controls produced no litters (Liu et al. 2013). These studies of resveratrol show that this polyphenol improves and maintains oocyte quality both in vitro and in vivo, respectively, and may be useful as a possible treatment for women experiencing sub-fertility issues. However, it is still unclear whether resveratrol must be supplemented throughout a female’s lifetime or if intervention during adulthood is sufficient to reverse aging phenotypes.

**Caloric restriction**

Restricting caloric intake to improve female fertility is not a new concept (Ball et al. 1947; Visscher et al. 1952; Nelson et al. 1985; Lintern-Moore & Everitt 1978; Masoro 2005). While continued dietary restriction reduces fertility, it can return once caloric intake is increased back to normal levels (Visscher et al. 1952; Selesniemi et al. 2008; Ball et al. 1947). Many studies have found that reducing calories upon weaning and later restoring an ad-libitum (AL) regiment can improve litter size and oocyte numbers in aged mice compared to control mice fed an AL diet throughout the lifespan (Ball et al. 1947; Visscher et al. 1952; Nelson et al. 1985; Lintern-Moore & Everitt 1978; Merry & Holehan 1979). Although these studies conclude that caloric restriction (CR) postpones the maternal age effect on fertility, it is unclear whether or not this is due to the stunted growth caused by CR initiated at weaning (Merry & Holehan 1979). To test this idea, Selesniemi et al. designed an experiment that introduced CR after the onset of sexual maturity in mice and reinstated an AL-fed diet at 15.5 months of age (2008). Once dietary restrictions were removed, aged mice previously on the CR diet showed marked increases in fertility rates, judged by the number of litters and survival rates of pups compared to age-
matched controls (Selesniemi et al. 2008). These results were similar to those obtained from younger mice in their reproductive prime (Selesniemi et al. 2008). This shows that caloric restriction still provides benefits to female fertility when induced later in life, and reproductive preservation is not solely due to the delay in puberty exhibited by initiating CR at weaning.

In addition to this study, Selesniemi et al. later analyzed oocyte quality a step further (2011). Previous research has shown that CR affects oocyte quality through metabolic pathways and oxidative phosphorylation, closely linked to mitochondrial function (Sohal et al. 1994; Barja 2002). Because mitochondrial function is closely linked to decreased oocyte quality with age, Selesniemi et al. examined chromosomal integrity, spindle formation, and mitochondrial aggregation in metaphase II oocytes of adult-onset CR-AL mice (2011). Compared to 12 month old AL-fed control mice, oocytes from 12 month old mice maintained on CR for 11 months and then AL-feeding for 1 month displayed spindle assemblies and rates of aneuploidy similar to 3 month old controls (Selesniemi et al. 2011). Although mitochondrial aggregation was increased in oocytes of 12-month CR-AL mice compared to 3-month controls, disruptions were not as severe as those seen in 12 month AL controls. Chromosomal misalignment increased in 3 month and 12-month control oocytes from 10% to 65%, respectively, but less than 25% of oocytes obtained from 12 month CR-AL showed the same abnormalities (Selesniemi et al. 2011). These data show that while the specific mechanisms by which CR improves fertility in aged mice are still unclear, it is likely due to increases in oocyte quality. It also remains unclear whether or not this method could be applied to humans, because this work suggests that CR would have to be initiated early on in adult life.

Conclusions
While the topic of declining female fertility with age is still a complex problem to be solved, recent research has made great strides in understanding how exactly female reproduction is affected by time. Oocyte quality plays a large role in the determination of future pregnancy success, yet the extreme variability observed in individual cases of infertility and subfertility indicate that other factors are at play (te Velde & Pearson 2002). The maternal age effect on reproduction clearly exists, but it is not yet completely understood which genetic and lifestyle influences are the cause of these observations with increased maternal age. Mitochondrial dysfunction has been closely linked with age and is a strong candidate for the cause of decreased oocyte quality with increased maternal age. As the major sources of ATP, mitochondria function is important for spindle formation and chromosomal segregation, two of the most important cellular events during oogenesis and embryogenesis (Battaglia et al. 1996; Kolano et al. 2012; Schwarzer et al. 2014; Fragouli & Wells 2011; Qi et al. 2014). Without a proper supply of functioning mitochondria, oocyte quality quickly decreases. Therefore it is important to further understand how mitochondrial function is affected by increased maternal age and how these issues may be addressed in the increasing population of older first-time mothers.

Because mitochondria contain their own DNA molecules separate from nuclear DNA, it would be beneficial to target gene expression and protein translation in aged individuals to determine the molecular differences between the two phenotypes. Mitochondrial DNA encodes only 13 proteins, while the remaining are imported from the nucleus (Schatz 1979). Differences in mitochondrial proteins may provide a clue as to which genes are up-regulated or down-regulated with age. Perhaps ameliorating missing or accumulated proteins with age with certain factors or enzymes would increase oocyte quality in older mothers. Researchers may also focus
on how to genetically modify mitochondrial or nuclear DNA within the oocyte to maintain longevity of quality with age.

The discovery of OSCs has created extensive opportunities for research into a new source of autologous oocytes perhaps less affected by maternal age than those generated during fetal life (Johnson et al. 2004; White et al. 2012). Although oogonial stem cells provide a continuous supply of immature oocytes during adulthood in mammalian females, the follicular pool is still exhausted with age to cause the onset of menopause. Increased understanding of the mechanisms behind how aging affects oogonial stem cells will surely shed new light in the field of female reproduction.

Clearly there is still much to be discovered about female fertility and its dynamics with age. Women are delaying childbirth further every year for various reasons, yet the biological clock remains unchanged. While more and more couples seek assisted reproductive treatments, it is still clear that there is no single treatment that is successful for every patient. Until the subtleties of female reproduction are uncovered, the ability to preserve fertility until later in life remains elusive.
Figure 1: An imbalance of mitochondrial biogenesis and mitophagy causes detrimental effects to the cell. This can be caused by an increased activation of one function without an increase in the other, or a decreased activation of one without the decrease of the other. If biogenesis is dominant, increased numbers of mitochondria cause increased ROS production, increased aggregation of excessive mitochondria, and an increased number of dysfunctional mitochondria due to the lack of efficient mitophagy. If mitophagy is dominant, the number of mitochondria will decrease, causing increased stress on the remaining mitochondria and a decreased level of ATP production overall.
Figure 2: Different kinds of chromosomal errors can cause aneuploidy. When chromosomes fail to recombine and form crossovers during prophase I, they do not line up together on the metaphase plate and can be segregated into the same cell. While there is a chance they can successfully separate into different cells, the lack of recombination between chromosomes can lead to increased risks for future aneuploidy. Premature separation of sister chromatids (PSSC) is the most common cause of aneuploidy. This results when one of the sister chromatids separates too early, leaving one homologous chromosome attached to a single sister chromatid. These can then go on to separate into the two cells as a single chromatid and three chromosomes rather than two sister chromatids in each. Nondisjunction describes the phenomenon during which homologous chromosomes or sister chromatids fail to separate at the appropriate times. This leads to both homologous chromosomes segregating into a single cell during meiosis I, or two sister chromatids segregating into a single cell during meiosis II, leaving the second cell with no chromosomes.

<table>
<thead>
<tr>
<th>Segregation error</th>
<th>Meiosis I outcome</th>
</tr>
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<tbody>
<tr>
<td>recombination failure</td>
<td><img src="image1" alt="Diagram" /></td>
</tr>
<tr>
<td>premature separation of sister chromatids</td>
<td><img src="image2" alt="Diagram" /></td>
</tr>
<tr>
<td>nondisjunction</td>
<td><img src="image3" alt="Diagram" /></td>
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Figure 3: Segregation of oocyte chromosomes during meiosis I and meiosis II creates a single oocyte and two polar bodies. Oocytes arrested in prophase of meiosis I contain two homologous chromosome pairs joined by recombination. After ovulation is induced by the LH surge, the oocyte resumes meiosis I and segregates each homologous pair into the oocyte and the first polar body. The oocyte proceeds through meiosis II and becomes arrested in metaphase II. Once, fertilized, the oocyte divides again to release the second polar body, and becomes haploid in preparation for fusion with the sperm cell.
Figure 4: Trisomy 21 is caused by three different types of segregation failure. Lack of crossovers prevents the exchange of genetic material between chromosomes and can lead to aneuploidy. Crossovers too close to the telomeres (distal recombination) or the centromeres (proximal recombination) can also lead to increases in aneuploidy that result in trisomy 21.
Figure 5: Cohesion proteins are necessary for the correct timing of chromosomal segregation. During meiosis I and meiosis II, homologous chromosomes and sister chromatids must be kept together until the exact moment of separation. If they segregate too early or too late, aneuploidy occurs due to PSSC or nondisjunction, respectively. Cohesion proteins form a ring around the ends of sister chromatids and the centromeres of homologous chromosomes. Cohesion surrounding the ends of the sister chromatids is first broken down during anaphase I, and cohesion around the centromeres is lost during anaphase II. This allows for proper segregation during cell division.
Figure 6: Methods of assisted reproductive fertilization. Two methods of fertilization in assisted reproduction are *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI). IVF involves the co-culture of oocytes with multiple sperm cells to achieve fertilization. Some couples require the use of ICSI, which involves injecting a single sperm directly into the oocyte.
Figure 7: Ooplasmic transfer causes mitochondrial heteroplasmy. In ooplasmic transfer, a small volume of young donor oocyte cytoplasm is injected into aged or poor quality oocytes during IVF procedures. This method appears to rejuvenate aged oocytes, but live offspring result in mitochondrial heteroplasmy. Because this was considered genetic modification, the FDA suspended the procedure in 2001.
Figure 8: Autologous germline mitochondrial energy transfer (AUGMENT). This procedure was inspired by the discovery of OSCs that supply renewable autologous mitochondria for women with poor oocyte quality. Transfer of mitochondria from these OSCs would supply the oocyte with new mitochondria, free of mtDNA damage that arises with increased maternal age.


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