The Effect of Acupuncture on Telomere Length and Sperm Quality: A Research Synthesis

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Abstract

Male infertility accounts for roughly 33% of the main causes of infertility among couples and is a major concern surrounding pregnancy outcomes and live birth rates. Current treatments are invasive and costly, and significant numbers of new findings and research suggest that these current forms of treatment such as Assisted reproductive technology (ART), Intra-cytoplasmic sperm injection (ICSI) and In vitro fertilization (IVF) have been shown to affect the fetus and cause problems later in life for those children. Acupuncture, which has been around for 5,000 years, holds promise as a treatment for sperm quality. Studies have shown that males have an increase in sperm count as well as faster movement of sperm with acupuncture treatment. Stress is a widely known factor that may affect male infertility because it relates to the aging process and overall decrease in general health and vitality. Telomeres, the end caps of chromosomes, are a general marker for a person's health, and their measurement may provide into the effects of stress on male infertility. Telomere length and telomerase levels are an important area of focus in medicine because studies are showing that measuring telomeres can show how a person is aging and also possible relationships between telomere length and cancer risk. The purpose of this study is to identify whether there is a correlation between the effects of acupuncture on telomere length, improvement in sperm quality and the ability of longer telomeres to improve sperm quality. This study also identifies the role of stress on factors related to male infertility and telomeres as well as how acupuncture may be shown to decrease stress. **Methods**: PubMed, Google Scholar, Yo San Library, EBSCO host databases and published books were explored for search terms including acupuncture, telomere length, telomerase levels, stress, male infertility and sperm quality. **Results:** Research revealed that male infertility and sperm quality are negatively affected by stress and acupuncture has been shown to minimize stress. In addition,

telomere length and telomerase were both shown to be inversely proportional to stress as well as sperm quality.

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Chapter 1: Introduction

Male Infertility

Definition of terms

Dohle et al., 2004, defined infertility as the inability of a sexually active, non-contracepting couple to achieve pregnancy within one year. The male partner can be evaluated for infertility using a laboratory evaluation of semen. According to Gnoth et al., 2005, a common definition of infertility is extremely useful for the suitable management of infertility. The term "infertility" may be used interchangeably with "sterility" with both resulting in only occasional occurrence of spontaneous pregnancies (Gnoth et al., 2005). The major factor affecting the individual spontaneous pregnancy prospect is the amount of time of undesirable non-conception, which determines the grading of infertility (Gnoth et al., 2005).

The scope of the problem

Dohle et al., 2004, state about 25% of couples do not achieve pregnancy within one year, of whom 15% of whom seek medical treatment for infertility and less than 5% of whom remain unwillingly childless. Schaeffer & Hotaling (2016) conceded that if just 15% of couples are infertile, with half due to male factor, then 7% of males have some degree of male factor infertility. Therefore, ten million men in this cohort aged 18–50 translates into 700,000 men with male infertility. When a man is infertile, difficulties are often related to sperm count or sperm quality. According to Gnoth et al., 2005, symptoms of male infertility include abnormally shaped sperm, sperm with poor motility, poor sperm quality, sperm that cannot attach the head to the egg and sperm that cannot penetrate the egg.

The quality of a man's sperm decreases with age (Gnoth et al., 2005). As a man ages, it takes longer for his partner to get pregnant (Gnoth et al., 2005). There's also an increased risk of not conceiving at all. The risk of miscarriage is higher if the father is over 45 (Neto, Najari & Goldstein, 2016). The children of older fathers are at greater risk of autism, mental health problems and learning difficulties (Neto, Najari & Goldstein, 2016). The average time to pregnancy if a man is under 25 is just over four and a half months but nearly two years if a man is over 40 (Neto, Najari & Goldstein, 2016). There is a five-fold increase in time to pregnancy if the male partner is aged over 45 years (Neto, Najari & Goldstein, 2016). For couples having In vitro fertilization (IVF), the risk of not having a baby is more than five times higher if the male partner is aged 41 or older (Taylor, 2003). The volume of a man's semen and sperm decrease continually between the ages of 20 and 80 (Taylor, 2003). The risk of miscarriage is twice as high for women whose male partner is aged over 45 than for those whose partners are under 25 (Taylor, 2003). Children with fathers aged 40 or older are more than five times as likely to have an autism spectrum disorder than children fathered by men aged under 30 (Taylor, 2003).

Epidemiology

Ashok (2015) found that the male factor infertility ranged from 20% to 70% and that the percentage of infertile men ranged from 2·5% to 12%. Infertility rates were highest in Africa and Central/Eastern Europe. Rates of male infertility in North America, Australia, and Central and Eastern Europe varied from 4.5-6%, 9%, and 8-12%, respectively (Ashok, 2015). According to the results, at least 30 million men worldwide are infertile with the highest rates in Africa and Eastern Europe (Ashok, 2015).

Known causes of male infertility

Gnoth et al, 2005, describe causes of male infertility in detail (See Table 1). Sperm production problems, blockage of sperm transport, erection and ejaculation problems, hormonal problems and sperm antibodies are the major known causes of male infertility (Gnoth et al., 2005). More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. (Jain, 2006). The remaining cases of male infertility can be caused by a range of conditions including anatomical problems, hormonal imbalances, and genetic defects (Jain, 2006). There are many causes of male infertility, which makes diagnosis difficult.

Table 1: Known Causes of Male infertility (Gnoth et al., 2005)

Sperm production problems	• Chromosomal or genetic causes • Undescended testes at birth • Infections • Torsion (twisting of testis in scrotum) • Varicocele (varicose veins of the testes)

	• Medicines and chemicals • Radiation damage • Unknown cause
Blockage of sperm transport	• Infections • Prostate-related problems • Absence of vas deferens • Vasectomy
Sexual problems (erection and ejaculation problems)	• Retrograde and premature ejaculation • Failure of ejaculation • Erectile dysfunction • Infrequent intercourse • Spinal cord injury • Prostate surgery • Damage to nerves • Some medicines
Hormonal problems	• Pituitary tumors • Congenital lack of Luteinizing hormone (LH)/ Follicle-stimulating hormone (FSH) as a pituitary problem from birth • Anabolic (androgenic) steroid abuse
Sperm antibodies	• Vasectomy • Injury or infection in the epididymis • Unknown cause

Diagnostic testing for male infertility

Taylor (2003) states that diagnosing male infertility problems usually involves the following:

- General physical examination and medical history, including sexual habits and sexual development during puberty.
- Semen analysis; semen is sent to a laboratory to measure the number of sperm present and to look for any abnormalities in the shape (morphology) and movement (motility) of the sperm, or any infections.
- Since sperm counts often fluctuate significantly from one specimen to the next, semen analyses are generally done over a period of time for the most accurate results.

Table 2 details frequent diagnostic tests used for male infertility.

Table 2: Diagnostic Tests Commonly Used for Male Infertility (Taylor (2003))

• Scrotal ultrasound to look for varicocele or other testicular problems.
• Hormone testing, since the pituitary gland, hypothalamus, testosterone and testicles are involved in sexual development and sperm production.
• Post-ejaculation urinalysis; sperm can travel backward into the bladder instead of out the penis during ejaculation (retrograde ejaculation).
• Genetic tests, usually for low sperm concentration. A blood test can test for changes in the Y chromosome, signs of a genetic abnormality and

diagnose various congenital or inherited syndromes.
• Testicular biopsy (by removing samples from the testicle with a needle) can determine if sperm production is normal, which then differentiates from a blockage or another possible problem with sperm transport
• Specialized sperm function tests to check how well the sperm survives after ejaculation, how well they can penetrate an egg, and whether there's any problem attaching to the egg.

Treatment options for male infertility

Available treatment options available for male fertility problems include surgery, medication, hormone treatments, use of donor sperm and various Assisted Reproductive technologies (ART) procedures (Taylor, 2003). The most common ART procedures currently used are In vitro fertilization (IVF), Intrauterine insemination (IUI) and Intra-cytoplasmic sperm injection (ICSI), which are defined in the definition of key terms section. Table 3 provides detailed treatment options for infertile males.

Table 3: Treatments for Male Infertility (Taylor (2003))

• Assisted reproductive technology (ART) treatments, such as Intrauterine insemination (IUI), In vitro fertilization (IVF), or Intra-cytoplasmic sperm injection (ICSI: a single sperm is injected directly into an egg),
• A varicocele can often be surgically corrected, or an obstructed vas deferens repaired. Prior vasectomies can be reversed.

• Treating infections of the reproductive tract with antibiotic therapy.
• Treatments for sexual intercourse problems, such as medication or counseling for erectile dysfunction or premature ejaculation.
• Hormone treatments and medications, such as injections of human chorionic gonadotropin and or recombinant human follicle stimulating hormone.

Shortcomings of current male infertility treatments

Current treatments for male infertility have serious shortcomings (Sylvest et al. 2016). Intrusiveness or complications of surgeries, for example, surgery to remove a varicocele, may include pain, blood clots, heart disease risk, prostate cancer and dementia risks (Sylvest et al., 2016).

Dohle et al., 2004 states that In vitro fertilization (IVF) is a method used by reproductive endocrinologists to bypass many of the male factor sperm issues. However, IVF can sometimes create complications for the unborn child (Dohle et al., 2004). According to Dohle et al., 2004, more than 12 scientific papers have been published in the past year suggesting a connection between fertility treatments and low birth weight infants or disorders such as hypospadias (penis opening on back instead of front), heart malformations, chromosomal abnormalities or other major birth defects. In 2008, the Centers for Disease Control (CDC) noted that babies conceived with IVF had a slightly increased risk of several birth defects, including a hole in the heart, cleft palate, improperly developed esophagus and malformed rectum. There may be some abnormal gene expression associated with IVF, which increases the chance of genetic disorders (Dohle et al., 2004). Advanced paternal age using IVF has been linked to autism, Marfan and Apert syndromes and other problems (Dohle et al., 2004).

Genetic causes of infertility

Infertility is a broad term used to define a range of different phenotypes. According to Shah et al (2003), "The genetic causes of infertility are varied and include chromosomal abnormalities, single gene disorders and phenotypes with multifactorial inheritance". The study also mentions that "specific genotypes and karyotypes have been associated with infertility and studies of specific genes in humans and model systems shed light on the nature of the polygenic and multifactorial basis of infertility" (p.17).

DNA fragmentation

DNA fragmentation may predict the success of ART. ICSI may be used when there is high DNA fragmentation of sperm (Najari et al., 2016). Rodriguez et al (2005), states, "sperm chromatin abnormalities or DNA damage can impact human male fertility" (p.843). Evenson et al (2002) relates to this idea in stating that "sperm cells with sizable DNA breakage are generally present in human ejaculate, but infertile males typically have a higher proportion of sperm cells with DNA fragmentation" (p.36). DNA fragmentation is the separation or breaking of DNA strands into pieces (Carrell et al., 2009). The genetic integrity of the spermatozoon is essential for normal embryo development (Evenson et al., 2002). A high level of DNA fragmentation in sperm cells may represent a cause of male infertility that conventional examinations - sperm concentration, motility analysis, and morphology assessment - cannot detect (Evenson et al., 2002). Regardless of the assisted reproductive technology used, elevated levels of DNA fragmentation above the critical threshold will significantly compromise the possibility of a successful pregnancy (Niederberger, 2016). A DNA fragmentation test is an effective method for measuring thousands of sperm in an ejaculate (Carrell et al., 2009). Sperm are stained with a fluorescent probe that interacts with the DNA molecule (Niederberger, 2016). The fluorescence signal changes when the DNA is fragmented, and this is monitored using a flow cytometer (Niederberger, 2016). Carrell et al., 2009, recommends that this test be done on all infertile males.

Table 4: DNA Fragmentation Index (DFI): Percentage of Sperm Cells Containing Damaged DNA (Niederberger, 2016).

• ≤ 15% DFI = excellent to good sperm DNA integrity (IUI), In vitro fertilization
• > 15 to < 25% DFI = good to fair sperm DNA integrity
• > 25 to < 50% DFI = fair to poor sperm DNA integrity
• ≥ 50% DFI = very poor sperm DNA integrity

Telomere Length Affecting Male Infertility

What are telomeres and telomerase?

Telomeres are the end caps of chromosomes (Rodriguez et al., 2005). They ordinarily shorten with each round of DNA replication, which ultimately leads to the inability of cells to divide, leading to cell death, according to Rodriguez et al., 2005. Telomeres are important because they protect the ends of our chromosomes, much like the plastic tip on shoelaces (Fajkus, Dvorácková & Sýkorová, 2008). If the telomeres were not there, our chromosomes may end up sticking to other chromosomes (Fajkus, Dvorácková & Sýkorová, 2008). Since chromosomes are shortened during each replication, the only part of the chromosome that is lost is the telomere, and the DNA is left undamaged (Fajkus, Dvorácková & Sýkorová, 2008). Without telomeres, important DNA would be lost every time a cell divides which would eventually lead to the loss of entire genes (Fajkus, Dvorácková & Sýkorová, 2008).

The chromosome telomere is a series of 5'-TTAGGG-3' repeats found at the terminal region of a chromosome (Rodriguez et al., 2005). According to Rodriguez et al (2005), “This repeat sequence plays a vital role in DNA replication and in the protection of the chromosome by inhibiting chromosomal fusions, and in chromosomal localization in the nucleus” (p.844). The length of the telomere varies between chromosomes and is shortened with every cell division by 50 to 200 base pairs (Rodriguez et al., 2005). Telomeres consist of the same sequence of bases repeated about 3,000 times and can reach up to 15,000 base pairs in length (Rodriguez, 2007). When the telomere becomes too short, the chromosome reaches a ‘critical length’ and can no longer be replicated, which triggers the cell to die, a process called apoptosis, also known as cell death (Rodriguez, 2007). Rodriguez et al (2005) state, “All cells with a nucleus contain chromosomes and telomeres and exclude mature red blood cells and platelets” (p.844).

Telomeres ordinarily shrink by 1% annually, from birth to death. The telomeres of people with unhealthy habits have increased shrinkage (Rodriguez et al., 2005). Those of people with healthy habits and genes shrink at a slower rate, therefore helping these people to possibly live much longer (Rodriguez et al., 2005). Ultimately, telomere health is a significant determinant of health and longevity (Shealy (2016). Rejuvenation or regrowth of telomeres is, therefore, a crucial key to longevity and health according to Shealy (2016).

Table 5 provides the testing methods to measure telomere length. Each test analyzes either DNA, metaphase chromosomes or interphase nuclei (Montpetit et al., 2009).

Table 5: Telomere Length Testing Methods (Montpetit et al., 2009)

• HT Q-FISH: High throughput quantitative fluorescence in situ hybridization
• MMqPCR: Monochrome multiplex quantitative polymerase chain reaction
• qPCR: Quantitative polymerase chain reaction
• STELA: Single Telomere Length Analysis
• TRF: Terminal restriction fragment.
• PRINS: Primed in situ subtype of Q-FISH
• Q-FISH: Quantitative fluorescence in situ hybridization

Telomerase is an enzyme (reverse transcriptase) with protein + RNA subunit, that adds nucleotides to telomeres, prevents telomere shortening and repeated cell death (Fajkus, Dvorácková & Sýkorová, 2008). According to Fajkus, Dvorácková & Sýkorová (2008), “Telomerase is expressed by germ cells, early embryonic cells and stem cells, but not expressed by most somatic cells” (p.273).

The action of telomerase allows cells to keep multiplying and avoid aging (Fajkus, Dvorácková & Sýkorová, 2008). According to Fajkus, Dvorácková & Sýkorová (2008), “Because the lost sequences of the telomeres at each replication cycle can be resynthesized by telomerase, the enzyme has essential functions for cell immortalization” (p.275). Telomerase is important because it reverses telomere shortening and allows each generation to replace lost DNA, which allows cells to divide without ever reaching a limit (Fajkus, Dvorácková & Sýkorová, 2008).

Telomere length and telomerase levels in sex (germ) cells v. in regular (somatic) cells and the significance of each

Chan & Blackburn (2004) state, “Telomere length has been demonstrated to both reflect and limit the replicative lifespan of normal somatic cells, any cell of a living organism other than the reproductive cells” (p.119). The replication of normal somatic cells is limited by telomere shortening, which additionally carries on with each round of cell division, resulting in the loss of 50–200 terminal base pairs of the telomere in humans both in vitro and in vivo (Lee et al., 2015). With ongoing rounds of DNA replication, the telomeres become shorter. However, telomerase can lengthen telomeres (Chan & Blackburn, 2004). Incomplete replication in the absence of telomerase results in growing amounts of telomere shortening, which leads to the DNA damage and cell death (Chan & Blackburn, 2004). Therefore, proper telomere length and telomerase activity are necessary for replication of cells (Lee et al., 2015).

Telomerase is only found in very low concentrations in our somatic cells, whereas it is found in high levels in germ line cells (egg and sperm) and stem cells (Rudolph, 2007). Cells that divide rapidly, such as germ cells and stem cells, are among the few cell types in our bodies containing active telomerase (Rudolph, 2007).

Telomeres, telomerase and cancer

Telomerase is also found in high levels in cancer cells (Shay et al, 2001). This enables cancer cells to be immortal and continue replicating themselves (Shay et al, 2001). According to Shay et al (2001), "If telomerase activity were switched off in cancer cells, their telomeres would shorten until they reached a critical length" (p.679). This would prevent the cancer cells from dividing uncontrollably to form tumors (Shay et al, 2001). However, blocking telomerase activity could affect cells where telomerase activity is important, such as sperm, eggs, platelets, and immune cells (Shay et al, 2001). Shay et al (2001) states, "Since telomerase activity in somatic cells is very low, these cells would therefore be largely unaffected by anti-telomerase therapy" (p.683).

Mu & Wei (2002) found that, "Telomerase activity may be detected in a variety of normal and malignant cell types by using a sensitive polymerase chain reaction-based method, known as the telomerase repeat amplification protocol (TRAP) assay" (p. 4). Wesch, Burlock & Gooding, 2016 reported that telomerase activity was present in almost all tumor samples but could not be detected in normal tissues, except for germ cells in the ovary and testis.

Telomerase activity is negative in human normal somatic cells but can be detected in most tumor cells (Mu & Wei 2002). Telomerase inhibitors might be useful in selectively killing cancer cells while saving normal cells (Wesch, Burlock & Gooding, 2016). According to Mu & Wei (2002), “The desired effect of telomerase inhibition would be to shorten telomeres to critical lengths, causing replicative senescence and preferably cell death due to irreparable chromosome damage. These objectives are feasible because the majority of tumors have shorter telomeres and higher proliferation rates than normal replicative cell populations, thus inhibition of telomerase activity may only be necessary over a few cellular divisions to attain lethal erosion of a telomere” (p.6). In contrast, Kim et al., 1994, found that the longer telomeres of stem cells and germ line cells should not lose their chromosomal protective function, allowing rather than inhibiting telomerase to prevent any telomere shortening.

Male infertility correlations to telomeres

Abnormal shortening of telomeres has been associated with male factor infertility (Inagaki, 2006). Sperm maintain longer average telomere length because telomerase can rebuild the sperm cells if they begin to shorten, but sperm telomere length varies among individual men and individual spermatozoa (Inagaki, 2006). Variation in sperm telomere length could result from the effects of oxidative stress, which shorten telomeres (Inagaki, 2006). According to Kalmbach et al (2013), “Compromised telomere length in sperm may contribute to segregation errors, apoptosis, programmed cell death, with reduced sperm count, and reduced fertility" (p.25).

Acupuncture and Male Infertility

Acupuncture involves the insertion of fine and thin needles into the skin at important points on the body (Pei et al., 2005). Traditional Chinese medicine explains acupuncture as a technique for balancing the flow of energy through pathways (meridians) in the body (Pei et al., 2005). By inserting needles into specific points along these meridians, the energy flow will go back to homeostasis, according to the Pei et al., 2005. Acupuncture is an optional/additional treatment modality that can be used to enhance male fertility (Pei et al., 2005). According to Pei et al (2005), "Acupuncture infertility treatment produces few or no side effects while performing similar functions as the drugs do by stimulating the hypothalamus to effectively balance the endocrine system and its hormones and to get to the root cause of male infertility" (p.143).

Chen et al (2011) states that "With acupuncture, sperm production and quality can be increased, mechanical blockages can be dissolved or reduced and hormonal factors can often be corrected by restoring balance to the entire body" (p.313). Sufficient energy and substances and unblocked semen pathway are all necessary for ideal sperm quality (Pei et al., 2005). Three organ systems that can be addressed with acupuncture treatments are 1.) Kidney for growth and development, 2.) Liver for free flow of energy and 3.) Spleen for energy, sleep and sperm production (Chen et al., 2011). According to Sklar (2016), one of the ways acupuncture increases fertility is by reducing stress. Sherman et al (1997) states, "Acupuncture infertility treatment counters the effects of stress and cortisol by releasing endorphins in the brain, creating a calming effect" (p.159). Acupuncture also increases fertility by strengthening the immune system, which can play an important role in conception (Sherman et al., 1997).

Stress

Nargund (2015) states, "Psychological stress can be defined as any uncomfortable 'emotional experience' accompanied by predictable biochemical, physiological and behavioral changes or responses" (p.373). When people are under stress, the hormone cortisol is released in the brain (Mayorga-Torres et al., 2016). According to Nargund (2015), "This alters the brain's neurochemical balance, changing hormone levels and disrupting the pituitary balance" (p.381).

How stress relates to telomere length and telomerase levels

Stress has been shown to decrease telomere length (Epel et al., 2004). Psychological stress is significantly associated with higher oxidative stress and shorter telomere length, which are known determinants of cell senescence and longevity (Zalli et al., 2014). According to Zalli et al (2014), "Higher levels of perceived stress decrease telomere length on average by the equivalent of at least one decade of additional aging compared to low stress" (p.4520).

How stress relates to male infertility

Stress is a major underlying factor in infertility (Reynolds, 2016). Because of the delicate balance between the hypothalamus, pituitary, and reproductive glands, stress can alter sperm counts, motility, and cause impotence (Mayorga-Torres et al., 2016). Emotional stress plays a detrimental role on fertility (Nargund, 2015). According to

Collodel (2008), “Data shows that, among sperm pathologies, necrosis and apoptosis were higher and the number of healthy sperm was significantly reduced in stressed men. The effects induced by stress also seem to include meiotic and structural alterations in sperm cells” (p.255).

McGrady (2009) looked at the effects of psychological stress on male fertility, showing that stress is associated with reduced paternity and abnormal semen parameters. Enough scientific evidence exists to suggest that psychological stress could severely affect spermatogenesis (McGrady, 2009). According to Collodel (2008), “The autonomic nervous system and the adrenal hormones participate in the classic stress response while also affecting the reproductive system. Evidence exists that mild-to-severe emotional stress depresses testosterone and perhaps interferes with spermatogenesis in the human male” (p.259).

The effect of acupuncture on stress

Pilkington et al (2010), show that “studies on the endorphins released by acupuncture can raise the amount of white blood cells, T-cells and anti-bodies, which increase the body's level of immunity” (p.92). More recent work by Chao et al (1999) supports the concept that “Acupuncture activates the endogenous opioids in the central nervous system, which, in turn, inhibit central sympathetic neural outflow” (p.2128). According to Pomeranz (1988), “Evidence that acupuncture increases central nervous system opioids includes early work in a mouse model of pain, in which electro acupuncture at the large intestine 4(Li4) site produced analgesia” (p.228).

Yao & Thoren (1982) show "Experimental evidence in animal models of cardiovascular disease supports the concept that cardiovascular effects of acupuncture are mediated by inhibition of central sympathetic neural outflow, perhaps through release of endogenous opioids" (p.77). Eshkevari et al (2012) found that "Electronic acupuncture blocks the chronic, stress-induced elevations of the HPA axis hormones and the sympathetic NPY (Neuropeptide Y), a protein-coding gene pathway" (p.19). Sun et al (2005), report that "Experiments during acute stress show that electro-acupuncture (EA) at ST36 blocks chronic stimulation of the hypothalamic–pituitary–adrenal axis (HPA), and thus might reduce the physiological effects of the acute stress" (p.4963). Eshkevari et al (2015) also note that "Stress-induced circulating ACTH and corticosterone (CORT), as well as central CRH mRNA and protein were blunted when EA at acupoint stomach meridian point 36 (St36) was performed before exposure to chronic cold stress. This strongly suggests that EA at ST36 may be a viable therapeutic modality for chronic stress" (p.3649).

Statement of Research Question and Hypothesis

The intent of this Capstone is to demonstrate the relationship between the effects of acupuncture on telomere length, improvement in sperm quality and the ability of longer telomeres to improve sperm quality. The role that increased psychological stress plays in this relationship is also discussed. The hypothesis of this researcher is that acupuncture can increase telomere length and sperm quality, and decreased stress levels in individuals can play an important role in positive outcomes.

Definition of Key Terms and Glossary of Abbreviations

ACTH: adrenocorticotropic hormone is a polypeptide tropic hormone produced and secreted by the anterior pituitary gland (Eshkevari et al, 2015).

ART: assisted reproductive technology is the technology used to achieve pregnancy in procedures such as fertility medication, artificial insemination, In vitro fertilization and surrogacy (Dohle et al., 2004).

Asthenospermia: is when less than 32% of spermatozoids move ((Siterman et al., 2000).

Azoospermic: is the complete lack of sperm in the ejaculate (Siterman et al., 2000).

BDNF: **brain-derived neurotrophic factor** provides instructions for making a protein found in the brain and spinal cord (Lin et al., 2015).

CORT: corticosterone is a 21-carbon steroid hormone of the corticosteroid type produced in the cortex of the adrenal glands (Eshkevari et al, 2015).

CRH: corticotropin-releasing hormone is a peptide hormone and neurotransmitter involved in the stress response (Eshkevari et al, 2015).

DNA fragmentation: is splitting the DNA into shorter pieces by endonucleolytic DNA cleavage at multiple sites (Rodriguez et al., 2005).

EA: electro-acupuncture is the application of a pulsating electrical current to acupuncture needles as a means of stimulating the acupoints (Gu et al, 2011).

FRTA: **free radical theory of aging** states that organism's age because cells accumulate free radical damage over time (Desai, Sabanegh, Kim & Agarwal, 2010).

Genotype: is the organism's full hereditary information (Esteves, 2013).

Germ cell: is any biological cell that gives rise to the gametes of an organism that reproduces sexually (Rodriguez et al., 2005).

HPA: hypothalamic–pituitary–adrenal axis is a complex set of direct influences and feedback interactions among three endocrine glands: the hypothalamus, the pituitary glands and the adrenal glands (Eshkevari et al, 2015).

ICSI: **intracytoplasmic sperm injection** is an assisted fertilization technique consisting of the microinjection of a single viable sperm into an extracted ovum (Siterman et al.**,** 2000),

Infertile phenotype: is the physical characteristic(s) seen in an individual that renders him infertile along with genotype testing of chromosomes (Esteves, 2013).

IUI: intrauterine insemination is a fertility treatment that involves placing sperm inside a woman's uterus to facilitate fertilization (Siterman et al., 2000).

IVF: in vitro fertilization is the direct handling and manipulation of oocytes and sperm to achieve fertilization (Siterman et al., 2000).

Karyotype: is the number and appearance of chromosomes in the nucleus of a eukaryotic cell (Esteves, 2013).

Knockout mice: are laboratory mice in which researchers have inactivated, or "knocked out," an existing gene by replacing it or disrupting it with an artificial piece of DNA. Knocking out the activity of a gene provides valuable clues about what that gene normally does (Lin et al., 2015).

Meiosis: is the process of cell division by which reproductive cells (gametes) are formed (Desai, Sabanegh, Kim & Agarwal, 2010).

NPY: neuropeptide y is a 36-amino acid neuropeptide that acts as a neurotransmitter in the brain and in the autonomic nervous system of humans (Eshkevari et al, 2012).

Oligozoospermia: is when sperm concentration is less than 15 million/ml (Dieterle et al., 2009).

OTA: oligo-teratoastheno-zoospermic is a condition that includes low number of sperm, poor sperm movement and abnormal sperm shape (Siterman et al., 2000).

Oxidative stress: is a disturbance in the antioxidant balance, leading to potential damage (Aitken, Smith, Jobling, Baker & De Iuliis (2014).

PESA: percutaneous epididymal sperm aspiration is a procedure where a fine needle is passed into the tubes leading out of the testicles, or the testicles themselves, to retrieve sperm (Bidouee, Shamsa & Jalali (2011).

Phenotype: is an organism's observed property, such as morphology, development or behavior (Esteves, 2013).

ROS: reactive oxygen species are molecules or ions formed by the incomplete one-electron reduction of oxygen (Desai, Sabanegh, Kim & Agarwal (2010).

Senescence: is the condition or process of deterioration with age (Allsopp & Harley, 1995).

Sham acupuncture therapy: is essentially fake acupuncture, and is used as a control in scientific studies to determine whether or not the effects of a treatment are actually due to acupuncture treatment (Lin et al., 2015).

Somatic cells: are all cells in the body except germ cells, which are egg and sperm (Dohle et al., 2004).

Spermatocyte: is a male germ cell derived from spermatogonia (Dohle, et al. 2004).

Spermatagonia: are cells produced at an early stage in the formation of spermatozoa, formed in the wall of a seminiferous tubule and giving rise by mitosis to spermatocytes (Gnoth et al., 2005).

Spermogram: is a test where the male partner produces a sperm sample by masturbation into a sterile container (Bidouee, Shamsa & Jalali, 2011).

Sperm morphology: is the size and shape of sperm and is one factor that's examined as part of a semen analysis to evaluate male infertility (Gnoth et al., 2005).

Sperm motility: describes the ability of sperm to move properly through the female reproductive tract to reach the egg (Gnoth et al., 2005).

Telomerase: is an essential Ribonucleoprotein Reverse Transcriptase essential that adds telomeric DNA to the ends of eukaryotic chromosomes (Esteves, 2013).

Telomere: is a terminal section of a chromosome which has a specialized structure and which is involved in chromosomal replication and stability (Esteves, 2013).

Teratozoospermia: is abnormal sperm shape (Siterman et al., 2000).

TH: tyrosine hydroxylase is the rate-limiting enzyme in catecholamine synthesis (Benavides & Piccione, 2003).

Upregulation: is a positive regulatory effect on physiological processes at the molecular, cellular, or systemic level (Lin et al., 2015).

Chapter 2: Review of Literature

Impact of Telomere Length on Sperm Quality

Abnormal shortening of telomeres has been associated with male factor infertility (Esteves, 2013). The functions of telomeres (as stated in Chapter 1) include protection of the genetic information encoded on the chromosome, localizing the chromosomes in the nucleus and supporting DNA replication (Esteves, 2013).

Per Esteves (2013) study, "Telomeres have been targeted as potential candidates to explain some infertile phenotype characteristics in males" (p.177). Esteves' study, a clinical appraisal of the genetic basis in unexplained male infertility, examined the relationship between genetics and unexplained male infertility. Esteves found that currently, few diagnostic tools are currently available for routine use and their usefulness is not yet completely clear.

Chromosomal defects are the most common genetic abnormalities in infertile males, accounting for 2.1-15.5% of cases. According to Esteves (2013), "Klinefelter syndrome, chromosome translocations, inversions and deletions fall in this category and the vast majority of affected individuals display severely compromised semen quality. Translocation carriers, however, may have varying sperm production phenotypes ranging from normal spermatogenesis to inability to produce spermatogonia. Chromosomal abnormalities in somatic cells can be detected by karyotyping" (p.180).

Like chromosomal defects, gene mutations are usually related to severely abnormal sperm production phenotypes (Esteves, 2013). According to Esteves (2013), "Micro deletions in the Y chromosome, mutations in the cystic fibrosis gene and mutations of the androgen receptor gene are classic examples of genetic abnormalities in this disease category.
There is also a risk of unbalanced gamete production that results in an increased risk of spontaneous abortion and unbalanced offspring. The most relevant clinical aspect involves the carriers of translocations in chromosome 21 as they are at risk of producing a child with Down syndrome" (p.182).

Esteves (2013) states that "Sperm aneuploidy assessment in couples with unexplained infertility experiencing either repeated IVF failures or recurrent pregnancy loss can be performed by FISH while mutations and polymorphisms are identified by specific gene sequencing and mutational analysis methods" (p.182). Many advances are currently being made and the use of new genetic technology may hold the key to more accurately diagnosing and treating men with unexplained infertility (Esteves, 2013).

Desai, Sabanegh, Kim & Agarwal (2010) studied a free radical theory of aging and its implications in male infertility with regards to telomere shortening in humans. They studied the effects of mitochondrial generation of reactive oxygen species (ROS) and aging on human spermatozoa and seminal antioxidants and found that germ cell telomeres may be partially responsible for telomere shortening, decrease in sperm count, decline in testosterone concentration, and decline in motility with aging. They propose that ROS generation has a central role in the pathophysiology of age related decrease in male fertility.

Aitken, Smith, Jobling, Baker & De Iuliis (2014) studied oxidative stress and its impact on male reproductive health. Most of the discussion focused on the impact of oxidative stress at the level of gamete (Aitken, Smith, Jobling, Baker & De Iuliis, 2014). According to Aitken, Smith, Jobling, Baker & De Iuliis (2014), “If the oxidative stress is earlier in spermatogenesis, there are consequences for fertility and the health and well being of the offspring. Severe oxidative DNA damage in germ cells entering meiosis will simply precipitate an increase in cell death” (p.36).

An increase in telomere length has been shown to enhance sperm quality (Aitken, Smith, Jobling, Baker & De Iuliis, 2014). The impact of paternal aging on telomere length via aging is associated with oxidative stress in the germ line and increases telomerase activity and telomere length increase in spermatozoa (Aitken, Smith, Jobling, Baker & De Iuliis, 2014). According to Aitken, Smith, Jobling, Baker & De Iuliis (2014), “If the paternal germ line has experienced an oxidative stress post-meiotically, when telomerase can no longer increase (as is typically the case in infertile patients) then telomere length in the spermatozoa will be abnormally short and the implications for the health of ART offspring, potentially serious” (p.38). These studies showed how increased telomere length enhanced sperm quality.

Rodriguez and Herrera both used mice to study how telomere length affects male infertility. Rodriguez et al. (2005) studied the influence of excessive telomere shortening, a well-known trigger of sperm DNA fragmentation in late-generation knockout mice. Telomerase RNA Component (Terc) knockout mice are laboratory mice in which researchers have inactivated, or "knocked out," an existing gene by replacing it or disrupting it with an artificial piece of DNA. Knocking out the activity of a gene provides valuable clues about what that gene normally does (Lin et al., 2015). DNA fragmentation levels in sperm cells were measured from wild type, early, and late generation telomerase null mice (Rodriguez et al, 2005). A minimum of 1,000 spermatozoa was assessed from each mouse (Rodriguez et al, 2005). Terc knockout mice had a six fold mean increase in the percentage of sperm cells with fragmented DNA. Lin et al (2015) study showed “critically shortened and highly eroded telomeres in Terc mice and found testicular atrophy and depletion of germ cells, possibly leading to chromosomal abnormalities. DNA fragmentation causes common infertility in males. This could genetically inactivate sperm cells with defective genomic constitution, preventing the development of offspring with an abnormal genetic background and thus resulting in decreased fertility” (p.36).

Effect of Acupuncture on Sperm Quality

Research has shown that sperm quality can be positively influenced by acupuncture**.** Siterman et al. (2000), in a pilot study, showed a positive effect of acupuncture on sperm count, especially on men with such low sperm counts (or no sperm) that they would usually require a testicular biopsy to extract sperm for use in an IVF cycle. Semen samples of 20 males were taken. A light microscope and a scanning electron microscope then examined the spermatozoa before and a month after acupuncture treatments. Seven of the fifteen men with no sperm at all produced sperm detectable by the light microscope after a course of ten acupuncture treatments ($p < 0.01$) i.e. enough sperm could be produced for ICSI to be performed without having a testicular biopsy. Specifically, a definite increase in sperm count was detected in the ejaculates of ten of the fifteen azoospermic patients and a marked but not significant improvement in the sperm counts of severely OTA (oligo-teratoastheno-zoospermic**)**, low number of sperm, poor sperm movement and abnormal sperm shape, of males following acupuncture treatment.

Bidouee, Shamsa & Jalali (2011) presented a case study with single azoospermic male in whom spermatogenesis occurred who had sperm in his ejaculate after two courses of acupuncture. PESA (percutaneous epididymal sperm aspiration) using a fine needle to retrieve sperm and ICSI procedures had been done with failed results. Twenty acupuncture treatments twice a week using sterile disposable stainless steel needles (0.25 × 25 mm and 0.25 × 50 mm) were administered for twenty-five minutes of needle retention to points for a deficiency of the kidneys (hormonal imbalance) and damp-heat syndromes (inflammation of the genital tract). Only twelve of these points were used at a time during each session. His next spermogram showed a sperm count of 10 million/mL, with 10% good and progressive motility and 60% normal shape, in the same lab. He then received a second course of acupuncture. His third spermogram, in the same lab, showed a sperm count of 18 million/mL, with 30% good and progressive motility and 60% normal sperm.

Siterman et al. (1997) studied a group of infertile men with abnormal semen analysis who were randomly divided into two groups; one group was given ten acupuncture treatments over five weeks, and the other group, no treatment. Significant improvements ($p < 0.05$) were demonstrated in the acupuncture group compared to the control group; in particular, improved motility and morphology were shown**.** Sixteen acupuncture treated subfertility patients and 16 control untreated subfertility males were examined Siterman et al, 2007).

Pei et al. (2005) studied a group of infertile men who had pathological semen analyses. They were treated with acupuncture twice a week for five weeks. A statistically significant increase after acupuncture in the percentage and number of sperm with no structural defects was demonstrated compared to the control group of patients who received no treatment. They concluded that male infertility patients could benefit from having acupuncture. Forty men with idiopathic oligospermia, asthenospermia, or teratozoospermia were studied. Twenty-eight of the patients received acupuncture twice a week over a period of 5 weeks. The samples from the treatment group were randomized with semen samples from the 12 men in the untreated control group (Pei et al, 2005).

Dieterle et al. (2009) randomized fifty-seven patients who had extremely low sperm counts into acupuncture and placebo acupuncture groups. After receiving acupuncture twice weekly for six weeks motility of sperm (but not overall count) was found to increase significantly in the acupuncture group. The authors conclude that the results of the present study support the significance of acupuncture in male patients with severe oligoasthenozoospermia. More evidence with larger trials needs to be accumulated before the efficacy and effectiveness of acupuncture in male infertility can be evaluated.

Chen et al. (2011) studied the effect of acupuncture-moxibustion therapy on sperm quality in 118 infertility patients with sperm abnormality. The team found that levels of an important enzyme in the semen, seminal plasma acid phosphatase increased, as did the sperm motility after acupuncture, and were statistically significant ($P<0.01$).

Impact of Acupuncture on Telomere Length

Yoshiaki Omura performed a series of studies in 1998, 2007 and 2010 on the acupuncture point St 36 on humans to test its possible effects on telomere length. Omura, Shimotsura, Ooki, & Noguchi (1998), found that acupuncture on St.36 on apparently normal subjects increased telomere length up to a maximum of more than two times prior to the treatment, depending on the method of treatment, but increases were frequently between 60% to 100%. Subjects with unusually low telomere length often had chronic degenerative diseases, while those having exceptionally high telomere length often had excellent physical conditions or mental acuity. (Omura, Shimotsura, Ooki, & Noguchi, 1998).

Lin et al. (2015) studied acupuncture's effect on telomerase response in the brain of mice using St 36. To investigate the telomerase response in the brain to acupuncture, brain-derived-neurotrophic factor (BDNF) levels were studied in three randomly assigned subgroups of mice, one receiving acupuncture (acupuncture subgroup), one-receiving sham acupuncture therapy (sham subgroup), and one-receiving no treatment (control subgroup). The mice were either telomerase-deficient (Terc) or normal, wild type (WT). The mice receiving acupuncture did so for four days at 30 minutes each at St 36. For the two sham groups, the sham point was set at a location approximately 3mm to the lateral side of the tail on the gluteus muscle following the same schedule. Acupuncture at St 36 for four days upregulated BDNF of the telomerase-deficient (Terc) mice, but that result was not seen in the normal, wild-type (WT) mice with normally functioning telomerase. The research team believed that using acupuncture increased telomerase activity through the activation of BDNF and its downstream, activating pathways in populations of patients who exhibit low telomerase activity, thus increasing telomere length (Lin et al., 2015).

Telomere shortening associated with increased risk of cancer

Omura et al. (2007) studied an acupuncture point named "True St 36", a name coined by Omura, since the location varied slightly from the actual St 36 point location. Omura studied the cells of cancer patients and the results showed reduction in telomerase levels of these cancer cells, while increasing telomere length in the normal somatic cells, and in cells that did not exhibit cancer (Omura et al, 2007). Most human cancers have short telomeres and express high levels of telomerase, whereas in most normal somatic tissues telomerase is absent (Shay et al, 2001). According to Shay et al (2001), "Tumors can arise from genetically instable cells with defective telomeres. Since telomere shortening is heavily associated with an increased risk of cancer during aging and chronic disease, the deficiency of telomere capping function adds to the induction of chromosomal instability and cancer initiation process" (p.683).

In their third study, Omura et al. (2010) studied the effect of acupuncture with electrical stimulation at St 36 on telomere length in breast cancer and Alzheimer's. The results over the course of 10,20 and 30 minutes treatments showed telomere length had drastically increased in size in the normal somatic cells that were unaffected by the cancer, whereas cancer-related markers and abnormally high β-Amyloid levels in Alzheimer's patients steadily decreased (Omura et al, 2010).

Impact of Acupuncture on Stress

Sparrow & Golianu (2014) noted that there is evidence that acupuncture decreases the stress response in both human and animal subjects. This study measured the blood pressure of 10 hypertensive patients aged 33-72 and was a retrospective, uncontrolled case study. The researchers found that acupuncture can increase heart rate variability (HRV) in the short term (minutes to hours), which created a relaxed and low-stress environment. Sparrow & Golianu, (2014) state that "HRV has been linked to other accepted and precise physiologic markers of stress and inflammation pertinent to acupuncture treatment, one of them being telomere length, suggesting that the relaxed and calming HRV response may be applicable to clinical outcomes" (p.291).

Kondo & Kawamoto, 2014) found that acupuncture and moxibustion can improve the psychological relationship between doctors and patients, especially when it is disturbed by a “game”, a dysfunctional interpersonal interaction that is repeated unintentionally. Kondo & Kawamoto (2014) sate that “Acupuncture and moxibustion are helpful in treating somatoform disorders based on fundamental meridian points that are considered to have effects on central, autonomic nervous, immune, metabolic, and endocrine systems to regulate the whole body balance” (p.7).

Nakamura et al. 2010, reported in a factor analysis of 197 patients that the attentive attitude of the therapist and proper touching by the therapist were significantly associated with a decrease in the subjective symptoms by acupuncture and moxibustion therapy, while the physical factors of the therapeutic stimulation were not. These results indicate that acupuncture and moxibustion therapy is a kind of psychotherapy, which may be of assistance in establishing the psychological therapist-patient relationship, rather than a physical therapy (Nakamura et al, 2010). It was recently reported that some of these meridian points have effects on the autonomic nervous system. (Kondo & Masazumi, 2014). For example, Ren 12 and ST 36 increase parasympathetic nerve activity (Liu, Xu &Chen 2012). In addition, some of the meridian points have opposite effects, which are contrary to other points. LI 11 and GB 21 increase sympathetic nerve activity (Knardahl, Elam, Olausson & Wallin, 1998 and Yook et al, 2009). Ngai & Jones 2013 found that UB 13 decreases sympathetic nerve activity.

Kondo & Masazumi (2014) note, “Since the acupuncture point combinations include meridian points with bi-directional regulation of the autonomic balance, they can be applied to various pathological conditions in a balanced manner” (p.7). Inoue and Yamauchi, 2008, Shea et al, 2008 and Tonhajzerova et al, 2010 showed that acupuncture reduced sympathetic and parasympathetic nerve activities in people with both chronic anxiety disorders and severe depression. Minagawa et al, 2010, found that UB 32 reduces heart rate without having an effect on heart rate, which might be effective for patients with palpitations without heart rate variability abnormalities. Homma (1984) found that UB 10, UB 18, Ren 6, Ren 12, GB 20, DU 12, KD 6, LI 11 and St 36 have been shown to decrease anxiety. Park et al (2010) found that "Acupuncture for St 36 decreased anxiety-related behavior, the serum corticosterone level, and tyrosine hydroxylase-immunoreactive expression, the rate-limiting enzyme in catecholamine synthesis, of rats under immobilization stress" (p.113). Chen (1992) found that "UB18, Ren12, St 36, GB20, GB21 LR14, and St 36 have an anti-depressive effect. Electro-acupuncture at St 36 and GB20 reduced the Beck Depression Inventory scales in subjects with psychosomatic or psychiatric disorders such as fibromyalgia, irritable bowel syndrome, chronic fatigue syndrome, primary insomnia, and obsessive-compulsive disorder, probably by enhancing release of serotonin" (p.280). Eshkevari et al (2012) found that ST25 prevented chronic stress-induced increases in the sympathetic peptide, neuropeptide Y(NPY).

Chapter 3: Methodology

The goal of this study was to explore whether telomere length affects sperm quality, whether acupuncture can increase sperm quality and whether acupuncture can therefore affect telomere length. This Capstone also sought to determine whether acupuncture's affect on sperm quality might be due to acupuncture's ability to reduce stress, which has been shown to affect sperm quality. This author's hypothesis is that acupuncture increases telomere length, which results in higher sperm quality.

Designation of Methodology

This study uses a systematic quantitative literature review focused on the following:

- Does telomere length affect sperm quality?
- Can acupuncture increase telomere length?
- Can acupuncture therefore affect sperm quality?
- Is acupuncture's affect on sperm quality due to its ability to reduce stress, or is there evidence that acupuncture's affect on sperm quality is independent of its ability to reduce stress?

A quantitative literature review was chosen as the methodology for this study. Pickering and Byrne (2013) define the benefits of systematic quantitative literature reviews for PhD candidates and other early career researchers. The review is systematic in terms of how papers are initially assessed for inclusion. By mapping the boundaries of the existing literature it is possible to identify gaps in the literature, determine where generalizations occur, and identify the limits of those generalizations (Pickering and Byrne, 2013). A hypothesis was developed, and then a systematic literature review was performed to compile and analyze information.

Search Strategy

1. Online search of medical journals and peer-reviewed articles were performed through PubMed, NIH, Ebsco AltHealthWatch and Google Scholar.
2. Search terms used included: acupuncture and sperm, acupuncture and infertility, acupuncture and telomeres, acupuncture and telomerase, acupuncture and telomere

length, acupuncture and male infertility, acupuncture and sperm quality, telomeres and aging, acupuncture and sperm quality, acupuncture and sperm count, telomeres and sperm count, telomeres and male fertility, telomerase and male infertility, telomere length and sperm count, and telomeres and sperm quality.

3. Research for this study was collected from the United States, Japan, China, Brazil, Germany, Spain and Australia.

4. The articles used were all in English.

5. Of the 147 articles researched, 41 were used that fit the inclusion criteria.

6. Aside from articles, published books were used for research purposes.

Inclusion and Exclusion Criteria

The study included research from 2000 or later. Human and animal studies were included. Articles in English were included.

Excluded from the study were non-peer-reviewed articles. Articles not in English were excluded. Articles older than 2000**,** were excluded, in order to focus on the most current research.

Data Collection

A data abstraction form was used to extract relevant information for the current study. Criterion assessed included, name of article, date of publication, research method used and any outcomes attained in regards to each of the ensuing topics:

A. How telomere length can affect sperm quality

B. How acupuncture can affect telomere length

C. How acupuncture can improve sperm quality

D. How psychological stress affects telomere length and sperm quality

Once this data was collected, it was arranged into topics and sub-topics. These topics included acupuncture, telomeres and sperm quality. The sub-topics included: telomeres and telomerase, telomere length and telomere levels in germ and somatic cells, telomeres, telomerase and cancer, stress relating to telomere length and telomerase, stress relating to male infertility and the effects of acupuncture on stress. When studies with animals were used, the type of animal model used was specified.

Human Subjects Ethical Consideration

This current study is a literature review synthesis. Therefore, there are no human subjects being used. In this case, no ethical considerations need to be taken into account. This research proposal was reviewed and approved by the Yo San University Institutional Review Board (IRB). A copy of the letter of approval is included.

Chapter 4: Results

Overview

This chapter will summarize the findings of the studies conducted on acupuncture, telomere length, sperm quality and stress. The studies were grouped into categories based on similar research article findings. These categories were: telomere length and sperm quality, acupuncture and telomere length, acupuncture and sperm quality and stress affecting sperm quality and telomere length. A comparison of various acupuncture points was used, and animal or human studies and control groups were noted.

Thirty-eight original articles were reviewed that studied telomere length and acupuncture, acupuncture and male fertility and telomere length and male fertility. Upon further investigation, a question regarding what other factors may affect the outcomes of these studies became apparent. As a result, this author added the effect of stress on telomere length and the effect of stress on male fertility to her literature review. Ten additional original articles on stress and telomere length and stress and male fertility were reviewed as well. From the research, five groups were categorized into tables. These tables were compared and an analysis was presented based on the results of the findings.

Impact of Acupuncture on Telomere Length

From the ten articles reviewed that addressed acupuncture's effects on telomere length, three directly showed that acupuncture positively affects telomere length. One of three of those articles also examined use of electrical stimulation on the acupuncture point. Omura, Shimotsura, Ooki &Noguchi (1998) found that acupuncture at St 36 on the cancer patients treated showed a decrease in telomere length in adenocarcinoma from 950 nanograms (ng) to 750 ng and a decrease in telomere length in small cell carcinoma from 770 ng to 600 ng. Normal subjects treated showed an increase in telomere length at least double and sometimes between 60-100%. The study conducted in 2007 by Omura, Chen, Lu, Shimotsura, Ohki & Duvvi, used "Press Needle Stimulation of Omura's St 36 repeated 200 times, 4 times a day to cancer patients, which reduced cancer cell telomere length by 600-1500ng and high Oncogene C-fos Ab2 and

Integrin Î ±5Î21 of 100-700ng BDORT units to close to 1yg (= 10â᷉’24 g) BDORT units with a significant reduction of asbestos and mercury (Hg) from cancer cells, while markedly reduced normal cell telomere of 1yg was increased to optimally high amounts of 500Â-530ng BDORT units” (p.60). The significance of these numbers indicated that the needle stimulation was measured to have reduced cancer cell telomere length, while have increased the telomere length of normal cells.

Other researchers, such as Lin, et al (2015) also measured St 36 acupuncture point in mice to look at its effects on telomere length. Lin et al (2005) results found “e Terc-/- group showed down regulated hippocampal BDNF expression compared with the WT mice and acupuncture at St 36 for 4 days upregulated BDNF, TrkB, p75NTR, Akt, and ERK1/2 in the DG and hippocampus of the telomerase-deficient mice, but that result was not seen in the WT mice with normally functioning telomerase” (p.36). This research concluded that the use of acupuncture in pathologies associated with telomerase deficiency might provide some benefit by activation of brain-derived neurotrophic factor. Of the seven remaining articles, the results ended up being too heterogeneous to draw a conclusion.

Table 1: Overview of Studies (Acupuncture and Telomere Length)

1st Author (year)	Subjects	Measured	Intervention	Acupuncture points used	Results
Omura (1998)	*30 normal subjects *1 cancer	Telomere length	Acupuncture	Omura’s ST 36	Acupuncture increased telomere length in normal

	subject				cells and decreased length in cancer cells
Omura (2007)	Cancer patients: 28 y/o female 72 y/o female	Telomere length	Acupuncture	Omura's ST36	Acupuncture can decrease telomere length in cancer patients
Omura (2010)	*69 y/o female with cancer *79 y/o male with Alzheimer’s	Telomere length	*TEM (Transcutaneous Electrical Stimulation) *Acupuncture	Omura’s ST 36	Increasing normal cell telomeres by Omura's ST36 stimulation by press-needle stimulation, and increasing normal cell telomeres by transcutaneous electrical stimulation of Omura's ST36
Lin (2015)	*Terc-telomerase deficient mice *WT-wild-type	Telomere length	Acupuncture	ST 36	The Terc-/- mice in all subgroups had downregulated hippocampal BDNF expression compared with that

					of the WT mice in all subgroups.

Impact of Telomere Length on Sperm Quality

From the twelve articles researched on telomere length and sperm quality, five showed a direct correlation between telomere shortening with decreased male fertility. Of those five articles, two were mice studies and three were human studies. Studies in mice have proposed that there is a safety mechanism which degenerates spermatocytes with diminished telomere length to prevent their maturation. Rodriguez et al (2005) found that "Terc knockout mice had a six-fold mean increase in the percentage of sperm cells with fragmented DNA, since these mice of G3 (generation 3) had critically short telomeres" (p.844). Herrera et al (1999) study showed that in different generations of mice, the average length of telomeres was significantly shorter in the 6th generation mice than the earlier generations. According to Herrera et al (1999), "Mice lacking telomerase activity can only be bred for approximately six generations due to decreased male fertility and to an increased embryonic lethality associated with a neural tube closure defect" (p.2956). Of the seven remaining articles, the results ended up being too heterogeneous to draw a conclusion.

Two of the human studies both examined free radical theory and its effects on sperm quality. Desai et al (2009) examined “reactive oxygen species (ROS), important signaling molecules in an organism's regulation of metabolism and inflammation and male infertility. Accumulation of ROS may be responsible for telomere shortening and subsequent decrease in sperm count, decline in testosterone concentration, and decline in motility with aging” (p.17). Aitken et al (2013) found oxidative stress affecting the male with telomere length in the spermatozoa being abnormally short and the implications for the health of ART offspring, potentially serious. Emery & Carrell’s (2006) review showed that, “the telomere checkpoint is leaky in spermatocytes. Additionally, fertilization with telomerase null sperm causes severe defects in the resultant embryos” (p.139). These observations might indicate that these sperm are dysfunctional as a result of short telomeres in mature sperm (Emery & Carrell, 2006). According to Emery & Carrell (2006), “The telomere plays a major role in chromosomal localization within the sperm nucleus and it is possible that abnormal localization could cause meiotic errors resulting in the presence of an abnormal number of chromosomes in the sperm” (p.140).

Table 2: Overview of Studies (Telomere Length and Sperm Quality)

1st Author(year)	Subjects	Male factor	Measured	Results
Rodriguez(2005)	**14 mice (WT ,G1 Terc, G3 Terc)**	**DNA sperm fragmentation**	**Telomere shortening**	**Critically short telomeres are associated with sperm DNA**

				fragmentation
Herrera (1999)	**18 G3 mice**	**Decreased fertility**	**Short telomeres**	**Increased male infertility associated with telomerase deficiency appear earlier in mice with short telomeres**
Desai(2009)	**Human male subjects**	**Decreased fertility**	**Telomere shortening**	**Increases ROS responsible for telomere shortening & subsequent decrease in sperm count & motility decline**

Aitken(2013)	**Human male subjects**	**Decreased fertility**	**Telomere shortening**	**Oxidative stress is a major pathological mechanism responsible for both male infertility and DNA damage in the germ line.**
Emery(2006)	**Human male subjects**	**Sperm abnormalities**	**Telomere length**	**Fertilization with telomerase null sperm causes severe defects in the resultant embryos.**

Impact of Acupuncture on Sperm Quality

Of the sixteen articles reviewed on the impact of acupuncture on sperm quality, six directly correlated acupuncture treatments with increased sperm quality. Siterman et al (1997) found that “patients exhibiting a low fertility potential due to reduced sperm activity may benefit from acupuncture treatment, with a fertility index increase significantly (p < or = .05) following improvement in total functional sperm fraction, percentage of viability, and total motile spermatozoa” (p.156). Siterman et al (2000) performed another study, but this time with men with low to no sperm counts and found and found detectable sperm after the acupuncture ($p < 0.01$). As a result, enough sperm could be produced for ICSI to be performed. Again, in 2009, Siterman et al, measured acupuncture results which improved sperm quality by decreasing the temperature of the testes. Siterman et al (2009) results found “Seventeen of the 34 patients with hyperthermia, all of whom had genital tract inflammation, had normal scrotal skin temperature; in 15 of these 17 patients, sperm count was increased” (p.205).

Pei et al (2005) found a general improvement of sperm quality, specifically in the ultra structural integrity of spermatozoa, after acupuncture treatment. In their case study, Bidouee et al (2011), treated a 31 year old male with a history of failed PESA + ICSI before acupuncture. The results showed he had a sperm count of 18 million/mL in his ejaculation, after two courses of acupuncture. His next spermogram showed a sperm count of 10 million/mL, with 10% good and progressive motility and 60% normal shape, in the same lab. Following this, he received a second course of acupuncture. His third spermogram, in the same lab, showed a sperm count of 18 million/mL, with 30% good and progressive motility and 60% normal sperm. In measuring severely low sperm count as well, Dieterle et al (2009) studied 57 patients who had extremely low sperm counts, to acupuncture and placebo acupuncture groups. After receiving acupuncture twice weekly for 6 weeks motility of sperm (but not overall count) was found to increase significantly.

Table 3: Overview of Studies (Acupuncture and Sperm Quality)

1st author(date)	Subjects	Study/Control	Intervention	Measured	P-value/ Results
p Siterman (1997)	32 males	16-acu 16-control	Acupuncture	Sperm parameters /quality	P<or=0.05
Siterman (2000)	40 males	20-acu 20-control	Acupuncture	Sperm count	P<0.01
Siterman (2009)	57 males	39-acu 18-control	Acupuncture	Testes temperature/ Sperm count	P<0.01
Pei (2005)	40 males	28-acu 12-control	Acupuncture	Sperm count	P<0.05
Bidou (2011)	31 y/o male	NA	Acupuncture	Sperm count	Acupuncture is a successful method of treatment for idiopathic and non-obstructive azoospermia.

Impact of Stress on Telomere Length

Of the four articles regarding the impact of stress on telomere length, two directly linked high stress to shortened telomere length. Epel et al (2004) found that higher oxidative stress shortened telomeres in cells cultured in vivo where the continual caregiving stress of the mothers who were studied was related to telomere length. The results associate shorter telomere length with prolonged psychological stress. Zalli et al (2014) found that shortened telomeres with high telomerase activity and psychosocial factors contributed to differences in stress both psychologically and socially. The short telomere length and high telomerase activity group had fewer means of social support and higher hostility measurements. Of the two remaining articles, the results ended up being too heterogeneous to draw a conclusion.

Table 4: Overview of Studies (Stress and Telomere Length)

1st author(date)	Subjects	Methods	Studied	Measured	P-value and/or Results
Epel (2004)	19-control 39-caregiving	Questionnaire / PBMC's	Telomere length	Stress	$P < 0.045$
Zalli (2014)	333-split into three	Questionnaire /stress	Shortened telomeres	Stress	$P = 0.046$

	median groups	**testing/ PBMC's Cortisol-blood & saliva**			

Impact of Stress on Male Fertility

Out of the five articles reviewed that addressed stress and male fertility, four demonstrated a correlation between these two factors, and anxiety and depression were factored under the umbrella of stress. Pilkington et al (2007) reported promising results in the management of situational and generalized anxiety using acupuncture. In 2010 they performed another study showing how depression and anxiety together constitute a significant contribution to the global burden of disease. The study found it was not possible to accurately assess the effectiveness of acupuncture for these conditions or the relative effectiveness of different treatment regimens. Studies have assessed changes in levels of neurotransmitters with some data to back this up. Errington et al (2011) found that statistically significant effects directly attributable to an acupuncture treatment lends weight to the use of acupuncture to significantly reduce the symptoms of anxiety disorders, using both human and animal subjects. Wang et al (2014) found that acupuncture improved behavior changes in rats. The objective of the study was to observe the impacts of acupuncture on various hormones and acupuncture was seen to have regulated the stress reaction. Sparrow & Golianu (2014) looked into how acupuncture may reduce stress over time. The results showed that patients tended to have an increase in their HRV(heart rate variability), a measure of physiologic stress, during treatment, after needling, and, in some instances, an increase in HRV over weeks to months. These results indicated a relative decrease in their physiologic stress following acupuncture over the next few months.

Table 6: Overview of Studies: (Stress[Anxiety, Depression] and Acupuncture)

1st author(date)	Research	Study/ Control	Measured	Intervention	Results
Pilkington 2007	10 randomiz-ed control studies	4-experimental 6-control	Anxiety	Acupuncture	Insufficient evidence from research on acupuncture in the treatment of specific anxiety disorders for firm conclusions to be drawn.
Pilkington 2010	Clinical review	NA	*Anxiety *Depressi-on	Acupuncture	Because of the heterogeneity of these studies in terms of the acupuncture interventions and controls used, comparative

					effects cannot be effectively assessed
Errington 2011	Literature review	NA	Anxiety	Acupuncture	Limited/inconsiste -ncy-provide consistent, evidence-based recommendations regarding the adequate dose required for acupuncture to have a therapeutic effect.
Wang 2014	Male Sprague-Dawley rats	58 male rats-5 groups randomized-varying acupuncture	Stress	Acupuncture	Acupuncture regulates stress response in rats
Sparrow (2014)	Case study	NA	Stress and heart rate variability	Acupuncture	Acupuncture reduces stress by increases heart rate variability

Chapter 5: Discussion

Summary of findings

The objective of this review was to review current literature related to the author's hypothesis that acupuncture increases telomere length, which results in higher sperm quality. Promising data from Omura's study in both 1998 and 2007 showed that telomere length was significantly lengthened when using acupuncture on humans. Lin in 2015 found telomere length had a correlation with male fertility, namely sperm quality. Chronic stress was studied as a strong variable that could affect telomere length and sperm quality. However, not much data was found on this specific correlation.

Study limitations

Initially, the author intended to analyze available research on acupuncture's affect on telomere length, acupuncture's affect on sperm quality and telomere length's affect on sperm quality. It soon became clear that there was not enough evidence on the effect of telomere length on sperm quality, nor on the effect of acupuncture on telomeres. In addition, the author realized that the ability of acupuncture to reduce stress may play a key role in the affect acupuncture has on fertility.

Most current research found on telomere length involves the process of how it affects aging. Also, very few articles could be gathered to correlate acupuncture's affects on telomere length. When finding articles on how acupuncture can affect stress, not much research was found for stress in itself, but more on anxiety and even then not much research was available.

Other studies were found relating to this review, but they included other variables, such as acupuncture and moxabustion. They were not included because they did not meet the inclusion/exclusion criteria.

Theory of implication

A large implication of theory is how the Western doctors, especially reproductive endocrinologists, can use acupuncture as an adjunct to treatment. This knowledge also empowers patients who are going through this tedious and often times emotionally heartbreaking experience. Woman many times feel depressed that they can't get pregnant and think there is something wrong with them. Since 33% of infertility is due to the male factor, the public should be made more aware of the many fertility treatment options, including less invasive approaches. This could have the potential to take a large burden off females, who statistically are the ones who receive the majority of fertility and acupuncture treatments.

Recommendations for additional studies

With the current studies of epigenetics and telomere length on the forefront (Niajou, 2009) traditional Chinese medicine and namely acupuncture need to be further investigated. Research on heightened stress depleting the immune system has shown that illness comes about when the body is not in homeostasis (Saretzki & Zglinicki, 2002). As TCM practitioners, we see most patients have a large decrease in psychological stress with acupuncture. Since there have been many studies on meditation's effects on telomere length as well as meditation's effects on stress level, (Speca et al., 2000), the author suggests that further studies on acupuncture's effects on telomere length should be done. In my practice treating patients as well as my own personal experiencing receiving acupuncture, I have observed that the body maintains homeostasis after acupuncture just as it would through a deep relaxing mediation.

Conclusion

This synthesis illustrated that acupuncture can improve sperm count. These enhancements in sperm parameters could possibly give rise to improved fertility outcomes including better embryo quality, increased pregnancy rates and higher live birth rates. Evidence was also found correlating decreased telomere length with increased male infertility. This research suggests that telomere length can be increased, by decreasing levels of chronic stress. Acupuncture has been shown to decrease chronic stress, although more studies need to be done to strengthen this idea. Further controlled studies need to be done to address specific male factor infertility such as sperm count, motility, morphology and DNA fragmentation.

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Appendix 1: Table 4

Prognostic accuracy of sperm DNA fragmentation to predict outcome after IVF/ICSI (Lopez et al, 2013)

Statistical characteristics	***Pregnant***	***Non-pregnant***	**P** ***value***
DNA fragmentation (%)	18.90±7.71	21.61±11.59	0.102
Area under ROC curve (95% CI)	0.546 (0.450–0.642)		
Cutoff (%)	25.5		0.029
Sensitivity (%)	86.2		
Specificity (%)	28.9		
Positive predictive value (%)	48.7		
Negative predictive value (%)	72.7		

Abbreviations: ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilisation; ROC, receiver operating characteristic.

Appendix 2: Table 5

Methods Used to Assess Telomere Length (Montpetit et al., 2009)

Method	Analyte	Measures		Resolution (kb)	Optimally Suited for Large Studies
		Average	Chromosome-Specific		
TRF	DNA	Yes	No	1.0[a]	No
qPCR MMqPCR aTLqPCR	DNA	Yes	No	?[b,c,d]	Yes
STELA	DNA	No	Yes	0.1[a]	No
Q-FISH	Metaphase chromosomes	Yes	Yes	0.15–0.3[a,b]	No
	Interphase nuclei (telomere)	Yes	No	0.15–0.3[a,b]	No
PRINS	Metaphase chromosomes	Yes	Yes	0.3[a]	No
	Interphase nuclei (telomere)	Yes	No	0.3[a]	No
Flow-FISH	Interphase nuclei	Yes	No	0.2–0.3[a]	No
HT Q-FISH	Interphase nuclei	Yes	No	0.2–0.3[b]	Yes

Note. aTL = Absolute telomere length. DNA = Deoxyribonucleic acid. HT Q-FISH = High throughput quantitative fluorescence in situ hybridization. kb = kilobase. MMqPCR = Monochrome multiplex quantitative polymerase chain reaction. qPCR = Quantitative polymerase chain reaction. STELA = Single Telomere Length Analysis, Universal STELA. TRF = Terminal restriction fragment. PRINS = Primed in situ subtype of Q-FISH. Q-FISH = Quantitative fluorescence in situ hybridization.

Appendix 3: IRB Proposal

Appendix 4: Curriculum Vitae

EDUCATION

May 2015-present (anticipated graduation April 2017)
Yo San University
* Doctoral candidate of Acupuncture and Oriental Medicine
    * Reproductive Medicine specialty

September 2000-2004
Emperor's College
* Master of Traditional Oriental Medicine, *Magna Cum Laude*

September 1995-December 1998
University of Florida
* Bachelor of Science, Psychology, *Honors*
* Pre-medicine full course work
* Business, Minor

PROFESSIONAL EXPERIENCE

December 2004-present
Romy Simone: Cosmetic & Medical Acupuncture, Santa Monica, CA
Acupuncturist

September 2012-December 2013
Chinese Healing Arts Center, Santa Monica, CA
Independent Contractor-Cosmetic Acupuncture Specialty

March 2010-September 2012
Gentle Journey Spa, Santa Monica, CA
Independent Contractor-Cosmetic Acupuncture Specialty

February 2008-March 2010
Tola Wellness Spa, Malibu, CA
Independent Contractor-Cosmetic Acupuncture Specialty

December 2006-February 2008

Piel Skincare Spa, Beverly Hills, CA
Independent Contractor-Cosmetic Acupuncture Specialty

December 2004-December 2006
Veronica Skincare Spa, Malibu, CA
Independent Contractor-Cosmetic Acupuncture Specialty

LICENSES

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* State of California Licensed Acupuncturist
* NCCAOM Accredited
* ACE Fitness Licensed

TEACHING EXPERIENCE

May 2015-present
Yo San University, Los Angeles, CA Qi Gong Instructor

October 2005-present
Cosmetic Acupuncture Seminars, Santa Monica, CA
Private one-on-one cosmetic acupuncture training

July 2006-present
Various conferences and presentations, Los Angeles, CA Guest Lecturer

January 2005-2013
Private lecture series on health and anti-aging, Malibu, CA Sole Lecturer

September-December 2013
Emperor's College, Santa Monica, CA Assistant Patient Supervisor to Dr. Kim

September-December 2013
Emperor's College, Santa Monica, CA Assistant Herb Dispensary Supervisor

August 2012
Emperor's College, Santa Monica, CA Assistant Clinic Supervisor

September-December 2008
Emperor's College, Santa Monica, CA Medical Qi Gong Instructor

September 2004
UCLA, Los Angeles, CA Medical Qi Gong Instructor

September 2002-2004
Emperor's College, Santa Monica, CA Medical Qi Gong Assistant Instructor

SPECIAL INTERESTS

Charity Sponsor:

January 2012-present
Unlikely Heroes, Los Angeles, CA

February 2013-present
Face Forward, Beverly Hills, CA

January 2014-present
Kevin Richardson, PAW Conservation Trust, Northeast South Africa

January 2014-present
The Jane Goodall Institute, South Africa

HOBBIES, INTERESTS AND ACTIVITIES

Argentine Tango, Gyrotonics, Show jumping-Horses, Electric Guitar, Surfing, Painting, Sculpture, Meditation, Yoga, Swimming, Ecstatic Dance, Radical Honesty