

**Effects of Traditional Chinese Medicine on Sperm Parameters with a Focus on DNA  
Fragmentation: A Literature Synthesis**

**A Capstone Project  
Submitted in partial fulfillment of the requirements for the degree  
Doctor of Acupuncture and Oriental Medicine**

**By**

**Virginia C. Prior, MATCM, L.Ac., Dipl.O.M.**

**Yo San University  
Los Angeles California  
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### Approval Signatures Page

This Capstone Project has been reviewed and approved by:

*Carola Gehrke*

*12/30/11*

---

Carola Gehrke, Ph.D., Capstone Project Advisor

Date

*Daoshing Ni*

*12/30/2011*

---

Daoshing Ni, Ph.D., L.Ac., Specialty Chair

Date

*Carola Gehrke*

*12/30/11*

---

Carola Gehrke, Ph.D., DAOM Program Director

Date

### **Abstract**

This project is a literature synthesis of research done on the possible effects of Traditional Chinese Medicine (TCM) on sperm parameters with a focus on DNA Fragmentation Index (DFI) in an effort to treat male infertility. Research studies and articles were gathered, summarized and qualitative data were extracted for analysis in order to investigate how acupuncture and Chinese herbal medicine may affect sperm, DFI and male infertility. Background and etiologies of DNA fragmentation, poor sperm quality and male infertility were examined. TCM was found to have a positive effect on semen parameters, hormone levels, immune markers and antioxidant levels, potentially facilitating fertilization, pregnancy and live birth rates in both natural conception as well as with the use of Assisted Reproductive Technology (ART). Larger, more controlled studies need to be done. Currently, there are no studies published on the potential effect of TCM on DFI, which might be a valuable investigation to pursue.

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## **Chapter 1: Introduction**

### **Background and Need for the Project**

#### **Male Factor Infertility**

Infertility is a common problem affecting 15% of couples in the United States. More than ever, couples are having trouble conceiving and are using Assisted Reproductive Technologies (ART) such as artificial insemination and in-vitro-fertilization (IVF). Infertility is a problem that affects the couple as a unit, regardless of the underlying etiology. Male reproductive dysfunction contributes to 40% of infertility, similar in incidence to female factors and yet is “largely ignored” (Simon et al., 2010). It is imperative that more research is conducted to better understand the pathophysiology of male infertility, elucidate novel, cost-effective, low-side-effect treatment strategies, improve outcomes and increase birth rates of healthy children (Simon et al., 2010).

#### **Semen Analysis**

The semen analysis (SA) has been traditionally used to evaluate male factor infertility and has long been considered the cornerstone tool. The most important parameters of the SA include total volume of semen, sperm count, motility (percentage of sperm that swim in a rapid progressive manner) and morphology (shape of the sperm) (Damone, 2008). Significant deficiencies in any of these parameters can lead to male factor infertility.

While useful, the SA has very significant limitations; it is an observational test, as opposed to functional, and may not be predictive of sperm abnormalities that are not visible under the light microscope. Therefore it is not the most sensitive test for assessing male fertility potential or determining genetic normalcy of sperm since it does not have the ability to measure functional parameters such as sperm DNA damage or chromatin maturity. Thus, a man may be infertile despite having a normal SA because of the presence of an undetected increase in sperm DNA damage. Furthermore, current strategies used to treat male factor infertility such as artificial insemination (IUI), In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI), a procedure whereby a single sperm is injected into the egg, focus on maximizing fertilization and do nothing to improve sperm or embryo quality (Agarwal et al., 2003; Ward et al., 2009).

### **Sperm DNA Integrity**

DNA integrity is essential for accurate transmission of DNA information. Not only does the sperm genome provide information in the form of the paternal DNA sequence, but it also provides a structural framework that includes centrioles and molecular regulatory factors that are required for proper embryonic development. DNA repair is essential for meiotic recombination and correction of DNA damage in both the germ cells as well as the proteins involved in all the major repair pathways expressed in the testis. Although all the mechanisms causing sperm DNA fragmentation are not completely understood, high levels of sperm DNA fragmentation are known clinically to lower rates of embryo

implantation, increase miscarriage rates, decrease live birth rates, and possibly have a long-term impact on the health of the offspring (Agarwal et al., 2003; Ward et al., 2009).

### **Measurement of DNA Damage: The Sperm DNA Fragmentation Index (DFI)**

Sperm DNA damage can be detected through various assays. DNA damage has been quantified using assays of DNA fragmentation. The most common and objective assay used to test DNA fragmentation is the Sperm Chromatin Structure Assay (SCSA). The main instrument in this test is the flow cytometer, which detects cells stained with a dye indicating damage. These cells are stained with a fluorescent dye and pass through a cylindrical glass structure, suspended in liquid, in high quantities. The semen is analyzed by a machine, which uses a laser to shine on the sperm. The sperm that present with a green color have low levels of DNA fragmentation. The sperm that present with a red color have high levels of DNA fragmentation. The machine reduces bias and reduces issues that come up with analyzing semen with the human eye under a microscope. The large numbers also make the exam more accurate because it is able to get a much broader sample and therefore more exact information about the sperm (Tantrikut et al., 2010).

DNA fragmentation is quantified using the DNA Fragmentation Index (DFI), which expresses the amount of fragmented DNA as a percentage of total DNA. Recent studies have shown that DFI measurements may be helpful in analyzing and treating male factor infertility, as sperm with levels of DNA fragmentation over 30% decrease the probability of achieving a successful pregnancy (Boe-Hansen et al., 2006). The DFI is being

incorporated more and more in the male fertility evaluation, but clinical indications for the specific tests have yet to be defined (Tantrikut et al., 2010).

The High Density Stainability test (HDS) quantifies the percentage of sperm with immature chromatin and immature proteins, while the Oxidative Stress Analysis (OSA) measures reactive oxygen activity. These tests provide secondary information about DNA damage (Tantricut et al., 2010).

### **Etiology of Sperm DNA Fragmentation**

There are many factors associated with sperm DNA fragmentation and infertility: prolonged abstinence, advanced paternal age (age over 46), smoking, toxin exposure (environmental or iatrogenic), any illnesses involving fever, leukocytospermia, cancer, varicocele, excessive heat exposure, radiation, testicular trauma and certain pharmaceuticals. Neuro-modulators and use of Serotonin reuptake inhibitors (SSRIs), oxidation and hormonal changes seem to be the most significant contributors to high DFI levels. A 5-week treatment with paroxetine (an SSRI) significantly increased the DFI in 35 male volunteers with normal pretreatment DFI (Tantrikut et al., 2010). The neuromodulatory effects of serotonin on the male reproductive tract theoretically slowed sperm movement, making them vulnerable to oxidative stress and increased DNA damage (Agarwal et al., 2003; Tanrikut et al., 2010; Werthman et al, 2007).

In 1998 Peter Schlegel, MD participated in the publication of an article published by Fertility Sterility stating that “the specific localization of eNOS [endothelial Nitric Oxide Synthase] to human spermatozoa suggests that nitric oxide may be involved in normal sperm physiology...however, aberrant patterns of sperm eNOS expression are associated with decreased sperm motility, possibly through the generation of excessive cytotoxic oxidants” (Schlegel et al., 1998). This article is one of many that explore the effects of oxidation on semen, which may help to explain why some anti-oxidants are may be helpful in improving sperm quality (Schlegel et al., 1998).

Oxidative stress from unchecked free radical peroxidation is thought to be the final common pathway leading to damaged spermatozoa. Moreover, growing evidence suggests that seminal oxidative stress is involved in many cases of idiopathic male factor infertility. Seminal reactive oxygen species levels can be readily detected and are an independent predictor of fertility potential (Tirado et al., 2010). Subsequent studies have shown that the use of anti-oxidant therapy can improve semen parameters and improve DFI. Oral antioxidant treatment appears to improve ICSI outcomes in those patients with sperm DNA damage (Agarwal et al., 2006; Greco et al., 2005; Lopes et al., 1998).

Another study on the effects of antioxidant use on DFI found that after antioxidant therapy testicular sperm samples had a decrease in DFI while ejaculated samples did not (Moskovtsev et al., 2010; Turk et al., 2007).

In terms of hormones (FSH, AMH and inhibin-B), sperm DNA damage and the markers of oxidative stress in infertility, it was found that DFI was 3-fold higher in male-factor

infertility, and negatively correlated with sperm motility. Moreover, a negative association between antioxidant activity and sperm concentration suggests that even minimal oxidative stress may influence sperm concentration. Although, in most cases there is no significant relationship between hormone concentrations, sperm DNA damage and total antioxidant capacity, suggesting other mechanisms for sperm dysfunction. However, in some studies, Estradiol has shown to have a protective effect against sperm DNA damage, while others show that certain environmental hormones and endocrine disruptors affect the male hypothalamic-pituitary axis and potentially cause infertility (Appasamy et al., 2007; Meeker et al., 2008; Sikka et al., 2008).

### **Traditional Chinese Medicine (TCM) Modalities and Sperm Integrity**

Sperm DNA is both exceedingly fragile, as well as important to conception. The etiologies and mechanisms of sperm DNA fragmentation are therefore very sensitive and mutable, and affected by even the smallest environmental shift (Agarwal et al., 2003). These etiologies are closely related to the components of Traditional Chinese Medicine (TCM). TCM is based on folkloric concepts that meridians run through the body and carry “chi” or energy. The theory holds that when this energy flow is blocked, disease occurs. In TCM there are 2 major modalities based on these theories that are commonly used to improve health and thought to increase fertility: acupuncture and herbal medicine. These modalities are fundamental to TCM. The herbal formulas used in TCM have been used for thousands of years and are gaining momentum in the field of integrative medicine. These modalities are thought to be synergistic and may alter neuronal, immune

and hormonal pathways. Preconception care for 3 months using Traditional Chinese Medicine modalities have been shown to have a positive effect on egg quality, and may have an effect on DFI in men known to have high levels of sperm fragmentation, possibly improving overall semen quality (Agarwal et al., 2003; Ward et al., 2009).

The therapeutic mechanisms of TCM may prove helpful in encouraging DNA sperm integrity and possibly decreasing DNA fragmentation by having a positive effect on oxidation, the immune system and hormonal balance. TCM modalities may also have a positive effect on sperm quality, improving conception, pregnancy, live birth rates and the result of a healthy child.

### **Summary: Research Question and Hypothesis**

This literature synthesis aims to find discoveries made in allopathic and integrative medicine which evaluate several TCM modalities and their effects on semen parameters with a special focus on DFI. The goal is to investigate the most current and novel ways of measuring sperm quality and DNA Fragmentation Index (DFI), as well as to investigate efficacious, non-invasive and new possibilities in treatment, such as TCM's most promising modalities: acupuncture and herbal medicine. Moreover, the results of this research synthesis may serve as a foundation and paradigm for improving gamete and embryo quality in couples trying to conceive naturally as well as those seeking Assisted Reproductive Technologies (ART). My personal interest in the topic is to become as educated as possible on the topic of male infertility, its causes, the best ways

of testing it, and finding new ways of treating it. More specifically, I chose to investigate this topic to discover if there has been any evidence that TCM has an effect on DFI levels, sperm quality or male infertility. My hypothesis is that TCM increases semen quality, lowers DNA fragmentation and increases the change of pregnancy outcome in couples who have been diagnosed with male factor infertility.

### **Glossary of Relevant Terms**

**Acrosin:** A proteinase (enzyme) contained in the acrosome on the head of a spermatozoa, involved in the penetration of the zona pellucida of the ovum by the spermatozoon.

**APA:** Advanced Paternal Age

**AsAb:** Antisperm Antibodies

**Asthenospermia:** Low sperm motility

**CHM:** Chinese Herbal Medicine

**DFI:** DNA Fragmentation Index

**Disomy:** The state of having two copies of a chromosome, in this article, referring to uniparental disomy, an extra chromosome from one parent.

**E:** Estrogen

**FI:** Fertility Index

**FSH:** Follicle Stimulating Hormone

**LH:** Leutinizing Hormone

**OAT:** Oligoasthenoteratozoospermia. Sperm that have oligospermia (low count or

number), asthenospermia (poor movement or motility) and teratospermia (abnormal or poor morphology or shape of sperm).

Oligospermia: Low sperm count

PRL: Prolactin

SA: Semen Analysis

T: Testosterone

TCM: Traditional Chinese Medicine

Teratozoospermia: Poor morphology in sperm.

## **Chapter 2: Review of Literature**

### **Overview**

Topics covered in this review will be an introduction to male infertility and how it is increasing in our society. Traditional ways of measuring sperm quality, such as the Semen Analysis (SA) will be examined, and more recent and controversial ways to measure semen quality, such as the DNA Fragmentation Index (DFI), as well as other emerging tests, will be examined. Etiology of DNA Fragmentation will be reviewed as well. Also reviewed will be TCM modalities (acupuncture and traditional Chinese herbal medicine) and their respective relationship with causes of male infertility, poor sperm quality, DNA fragmentation and pregnancy/live birth rates if available.

### **Resources Engaged**

Material for the Literature Review was gathered by primarily utilizing search engines such as Google Scholar, Elsevier and PubMed, as well as the UC California Library system. More specifically, key search words and phrases related to the topic to find articles and studies applicable to the study were used such as “Sperm Quality”, “Semen Analysis”, “DFI”, “Acupuncture + Male Infertility/Semen Quality/DFI”, “Herbal Medicine + Male Infertility/Semen Quality/DFI”.

## Review of Literature

### Background: The Increase of Male Infertility and Potential Causes

Males may be more vulnerable to environmental factors, and in some ways may be the weaker sex (Deadman, 2008). Male fetuses are miscarried and stillborn more than females, males have a higher rate of developmental problems, as well as a higher rate of disease and a shorter lifespan. In terms of reproductive health, men have had a 50% increase in testicular cancer, prostate cancer and a significant increase in cryptorchidism and hypospadias. There has also been a decline in the male birth rate as well as a “probable decline in sperm densities in many regions” (Deadman, 2008).

Sperm counts started to fall in the early 90’s, or at least started getting noticed for a significant decline as the British Medical Journal started publishing reports. Later, in 2007, a study was published in *Andrology* stating that “a 29% decline in sperm counts [took place] in Aberdeen between 1989 and 2002 - a period of just 13 years, with average sperm counts falling from 87 million to 62 million sperm per ml” (Sripada et al., 2007).

According to Deadman (2008), major contributing factors that are likely reasons for declining sperm count could be lifestyle changes or endocrine-disrupting chemicals in the environment, such as estrogen, xeno-estrogens (such as PCBs or PEs), anti-androgens (such as phthalates used since the 1930’s, and which accumulate bodyfat) and specific toxins, such as methoxychlor (an insecticide) and vinclozolin (a fungicide). Several

studies have shown that these chemicals are higher in infertile men, and are clearly correlated with “higher-than-expected numbers of abnormalities in human genital development” (Deadman, 2008), many of which are associated with reduced fertility. Most disconcerting is Deadman’s review of the Anway et al. (2005) animal study which found that some chemicals, which cause infertility in male offspring of pregnant animals that were exposed to the chemicals, also stay in the system for more than one generation. They can “cause damage that is passed on through at least four subsequent generations without further exposure, implying lasting genetic damage” (Deadman, 2008).

Deadman (2008) discusses an exhaustive list of major possible factors, discussed in this paragraph, including diet. Soy, for example, is also thought to reduce sperm concentrations. A 2007 study shows that men who consume soy have 41 million sperm/ml less than men who do not eat soy (Chavarro et al., 2007). A 2005 Belfast study also suggests that men who have soy have lower sperm quality (West et al., 2005). Body weight is also a factor, and as this is on the rise in the U.S., it may be an important factor. Being both underweight and overweight negatively effects sperm concentration (Jensenet et al., 2004). Also, high BMIs are associated with decreasing levels of testosterone (Jensenet et al., 2004). Men with genitourinary infections such as chlamydia and mycoplasma genitalium have 3.2 times more DNA fragmentation, 80% more physical abnormalities and 10% less motility (Gallegos et al., 2007). One 2007 study found that a 4-month course of antibiotics improved DNA fragmentation and increased pregnancy rates (Gallegos et al., 2007). Diabetes, also on the rise worldwide, is associated with higher amounts of DNA fragmentation (Agbaje et al., 2007). Lifestyle factors that

influence scrotal temperature also decrease spermatogenesis, such as laptop computer use, saunas and hot baths, use of tight or synthetic underwear, and working in hot areas (Sheynkin et al., 2005). There is some discussion about iodine and its connection to thyroid disease and sperm count, but so far nothing significant was found (Crissman et al., 2000). Other factors which are negatively associated with male subfertility include cryptorchidism, smoking, excessive alcohol consumption, excessive cannabis use, intense exercise, use of SSRIs, prolonged cycling, mobile phones positioned near the reproductive organs, repeated infection and surgeries. Antisperm antibodies may also contribute to male factor infertility, as well as female infertility (Deadman, 2008).

### **Male Infertility: Contributing Factors**

#### ***Advanced Paternal Age***

Due to societal changes, couples are putting off family building, especially in the West. This trend, in combination with unhealthy, sedentary lifestyles, may not only be detrimental to overall health, but specifically to reproductive health for both women and men. This may also prove significant as some women mate with men who are the same age or older than themselves. A 2003 study by Eskenazi et al., which Deadman reviewed (2008), found that semen volume falls by 0.03 ml per year of age after 22 years, motility declines 0.7% and motility decreases by 3.1%, and total progressively motile sperm count decreases by 4.7% (Deadman, 2008), showing that advanced paternal age may be an important factor.

One study, which took into consideration age, ethnicity, lifestyles, etiology, traditional sperm parameters and DFI, found that the trend of delaying fatherhood in men undergoing IVF/ICSI treatment is detrimental to sperm quality. This trend may be, in the West, one of the underlying contributors to male infertility (Hammiche et al., 2011).

Age, in most cases, tends not to affect implantation rate, except in oligozoospermic (low concentration) patients, in which case the chance of pregnancy decreases “5% for each year of paternal age” (Ferreira et al., 2010). Age may, however, affect fertilization, or the health of the embryo, which may down the road affect miscarriage rate. Overall the decline in semen parameters (volume, concentration, motility and morphology) in aging men may be due to morphological changes in the testes decreasing spermatogenesis (both qualitatively and quantitatively), and this could possibly be due to reduced blood Testosterone concentration, common in advanced age (Ferreira et al., 2010).

### ***SSRIs***

Antidepressants (specifically the SSRI paroxetine) may decrease sperm quality and fertility potential when measuring DNA fragmentation with the TUNEL assay, although, the standard semen analysis (SA) does not necessarily show any changes. Therefore, measuring the DFI may be an important measurement in this instance. Although DNA damage “may exist independent of standard semen parameters, the degree of DNA fragmentation correlates with poorer fertility and pregnancy outcomes, even when

techniques such as in vitro fertilization and intracytoplasmic sperm injection [ICSI] are applied” (Tanrikut et al., 2010). Not only does DNA integrity effect intrauterine insemination (IUI) but it also affects pregnancy outcomes with IVF and ICSI. Secondly, men who are trying to conceive should discontinue SSRIs for the duration of preconception care, as both sexual function and fertility potential may be adversely affected by the changes in sperm DNA integrity (Tanrikut et al, 2010).

From a cultural perspective, this is important. Over 10% of men in the U.S. are affected by depression, anxiety and OCD, which are all treated much of the time with SSRI’s, as the side effects are perceived as safer than other medications and treatments. Moreover, SSRI dispensing rates have risen over the years, reflecting the increased stress levels in our society, which may also have an effect on semen quality, at least on an epigenetic level (Tanrikut et al., 2010).

Past studies have shown that once men discontinue use of SSRIs their sperm quality increases again, showing that SSRIs inhibit motility, not volume, which would take several months to recover. The fact that ejaculatory and emission function are compromised as well may also contribute to low sperm motility during use of SSRIs (Tanrikut et al., 2010).

Also, hormone levels seem to be greatly affected while taking paroxetine. Serum T (testosterone) and E2 (estradiol) decreased with the use of this SSRI according to a recent 2010 study, although it was noted that in other studies using different SSRIs (such as

fluoxetine) serum T and E2 levels did not change. No changes in FSH levels were noted, further suggesting that the fragmentation was caused by transport issues rather than sperm production (Tanrikut et al., 2010).

### ***Hormones***

It's known that hormone levels play a role in initiating and maintaining male reproductive function, but to what extent and in what relationship with each other still needs to be explored. Moreover, disruptions and compensatory mechanisms in the hypothalamic-pituitary-gonadal axis complicate matters. Overall it had been found that FSH and LH are inversely proportional to poor sperm quality; ie, high levels of FSH and LH are often correlated with poor morphology, along with decreased levels of inhibin B (Meeker et al., 2007). High levels of inhibin B and free T4 were positively associated with concentration. Testosterone, which facilitates spermatogenesis locally in the Sertoli cells as a paracrine agent (and also provides feedback for GnRH and LH secretion) tends to increase sperm motility. In contrast, LH was found to decrease sperm concentration, motility and morphology, both of which may be related to the regulation of the HPG axis. In conclusion, FSH, LH, inhibin B, testosterone and free T4 are associated with semen parameters (Meeker et al., 2007).

### ***Endocrine Disruptors***

According to Sikka and Wang (2008), endocrine disruptors may include environmental toxins, heavy metals, pharmacological agents such as radiation, and biological factors such as aging, toxin exposure, physical injury, and chronic disease states. Specific endocrine disruptors, such as PCBs, DDT, dioxin, pesticides, etc. are similar in structure to estrogen and are anti-androgenic chemicals in the environment. As they may mimic some natural hormones, they may confuse the regulatory function of the endocrine system, and potentially have hazardous effects on the male reproductive axis. Many estrogenic pollutants have significant consequences due to multiple routes of exposure, widespread presence in the environment and their ability to bioaccumulate and not readily biodegrade. According to Sikka and Wang (2008), several well-controlled clinical studies have confirmed an increase in male-factor fertility (Sikka & Wang, 2008). Also, “newer tools for the detection of Y-chromosome deletions have further strengthened the hypothesis that the decline in male reproductive health and fertility may be related to the presence of certain toxic chemicals in the environment” (Sikka & Wang, 2008). There are three main mechanisms which currently explain DNA damage in spermatozoa: abnormal chromatin packaging, oxidative stress and apoptosis. Sikka and Wang (2008) state that “High levels of DNA damage means that sperm are less likely to undergo apoptosis which is a natural self-destruct process designed to rid the body of damaged cells. However, it is not clear whether increased damage arises because of chronological age or because of longer-term exposure to environmental factors that may cause such damage.”

More studies need to be done on environmental hormone disruptors, and the combination of chemicals in the system. There is a variety of internal and external factors that can cause testicular toxicity. More studies need to be done on whether the environmental causes out-weigh other influences such as glandular infection, nutritional deficiencies, aging, ischemia, oxidative stress, androgen insensitivity and the role of chronic inflammation on the reproductive organs, which is not completely understood as it may be asymptomatic and difficult to diagnose (Sikka & Wang, 2008).

Overall, spermiogenesis may be impaired by a certain history, disease, treatment or environmental factor, but the most important factor is genetic origin, especially when a single abnormality is dominant. The known fact is that the vulnerability of the human testis to physical, chemical, or psychological (stress) factors suggest that analysis of morphology can serve as a valuable indicator of the impact of toxic or genotoxic effects of occupations, lifestyle or environmental factors in the testis (Auger, 2010).

### **Testing Semen Quality**

*“Seeing if, and how, sperm are capable of confessing their errors or flaws externally.”*

*(Barratt et al., 2011)*

Testing a man’s semen is not only important to see what the cause of his infertility is, and better treat it. It is also important because if an infertile man chooses to do ICSI, which

“now provides fertility in many cases of severe idiopathic spermatogenic failure and obstructive azoospermia” (McLachin & O’Brian, 2010), he may be unknowingly using chromosomally defected sperm, possibly passing along fertility defects, or other congenital issues, in male offspring. “Infertility is associated with higher rates of aneuploidy in ejaculated or testicular sperm and increased chromosomal defects in ICSI offspring” (McLachin & O’Brian, 2010), and consistent testing is important. More specifically, azoospermic factor deletions are associated with low levels of sperm in both ejaculate as well as testis biopsies (McLachin & O’Brian, 2010). When these sperm are “available and used for ICSI, fertility defects in male offspring seem inevitable. Complete evaluation must be made so that affected couples can become informed about the implications of such issues within the context of assisted reproductive technology outcome and their potential offspring” (McLachlin & O’Brian, 2010). This presents the idea that no matter what route a man chooses to take in his path to treating infertility, examining it and treating it as best as he can before engaging in trying naturally or with ART may prove a wise idea (McLachin & O’Brian, 2010).

### ***Semen Analysis (SA)***

Currently, Semen Analysis (SA) is the only tool to assess male factor infertility, although it is limited in its ability to predict a male’s capacity to initiate a pregnancy. ICSI and IVF help in the process of conception, and are considered somewhat of a “cure”, but nothing currently exists to help specialists better understand the physiology of sperm and the potential for sperm to create a healthy pregnancy, especially with large groups of

“healthy” males not being able to impregnate healthy females. Currently, there is an ongoing search for an improved way to diagnoses and assess male factor infertility. Overall there seems to be no single factor to predict male factor. However there seem to be several factors involved in sperm function and molecular features that may serve as a potential marker as they affect the necessary parameters, embryo quality and assisted reproductive success (Garrido et al., 2008).

### ***Manual for Semen Analysis by the WHO***

The Strict method, recommended by the latest WHO manual for semen analysis, assesses the percentage of “ideal” morphology of spermatozoa and is recommended to be a defining test in male factor fertility. In reality, a very low percentage of spermatozoa in the semen of fertile men are actually “ideal” (Auger, 2010). Furthermore, there is a clear association between structure and function in a normal spermatozoon which has successfully travelled to the cervical mucus (Auger, 2010). Though within a native sperm sample, the sperm may be normally shaped despite an axoneme anomaly resulting in cell immortality, or a perfectly oval-headed sperm with normal texture may not have mature chromatin, or may have DNA fragmentation (Auger, 2010).

The latest WHO manual suggests the usefulness of focusing on abnormal morphology by helping to improve photographic examples of abnormalities and by developing various indices of teratozoospermia, as this may have a prognostic value for fertility, both in vivo and in vitro (Auger, 2010). Though some data show that natural fertility may have a

stronger association with abnormal morphology than with normal morphology; of course more studies need to be done (Auger, 2010). The most controversial aspect of morphology analysis is the fact that fertile men have a low percentage of normal morphology, at least based on current reports (Auger, 2010). More specifically, dysfunctional cells have been found in men with “normal” semen parameters, while normal sperm function has been found in men who were diagnosed with oligozoospermia (Barrat et al., 2011).

The WHO manual also states that sperm cells with subnormal or borderline features be considered abnormal, while some investigators state that in examination of the past and current research, there is no evidence based data stating that small variations in size, shape or texture is correlated with fertilization potential (Auger, 2010). Moreover, in France, the criteria for measuring sperm morphology are the same as the Strict method, except that all of the subnormal aspects are put in the normal category. In other words, some andrologists consider subnormal sperm as normal sperm (Auger, 2010).

In terms of clinical practice, it has been observed that some technicians do not use the detailed lab methods even when provided for them in a comprehensive manual, and that training exists, but seems to not get used or does not help that much. Another problem that some scientists have is that both scientists and clinicians denigrate the value of semen assessments based on “false assumptions”, such as the assessments are performed inadequately, which is incorrect according to Barrat et al. (2011).

“The evidence from external quality control schemes demonstrates that some patients will be referred for inappropriate treatment, e.g., ICSI when they may not even need ART. In simplistic terms, we are exposing a large number of couples to inappropriate financial and psychological stress. Additionally, we are exposing the female to harmful procedures, e.g., IVF. Perhaps this third lesson will not be executed until legal action ensues” (Barrat et al., 2011).

The main idea may be that the WHO’s newest method for semen analysis is correct, and what is needed are not new studies for a new manual, but new assessments of male reproductive potential (Barratt et al, 2011).

Although an essential tool to disseminate good practice in the field of male factor infertility and andrology, the tests seem to have poor prognostic power and show little about the causes of subfertility. How we diagnose male factor infertility needs to be revisited. One’s fertility is an ever-shifting state and “should be regarded as a continuum” (Ford, 2010). Ford also suggests, “the artificial binary division between fertile and infertile should be abandoned” (Ford, 2010). These potential new paradigms may be of great help in terms of practice, and the couples’ decisions. The more specific testing can get, the better. The ability to identify specific tests which detect underlying pathologies can help lead to more specific treatment. Ford suggests continuing the exploration of oxidative stress and intracellular regulation. And most recently, the investigation of proteomics may hold some insight (Ford, 2010).

## **Other Tests**

### ***Sperm Function Testing***

Sperm function testing used to be performed more regularly before a couple moved on to ART, in an attempt to find the issues for both partners. This fell out of favor, on an international level. The WHO's Laboratory Manual for Examination and Processing of Human Semen now groups these tests under "Research Procedures", which suggests that within the field of ART, health-care providers have switched from a more comprehensive view to a view which looks mainly at the female partners, as IUI, IVF and ICSI can bypass some of the issues within male factor. The underlying impression is that there is no reason to spend the time doing blood work or testing sperm when you can simply fix the couple's infertility by treating the female partner with hormones she may or may not actually need to conceive, or with expensive treatments such as ICSI (Lamb, 2010). "In part, this reflects the current clinical practice of bypassing the in-depth evaluation of the male partner, while assuming that if a spermatozoon can be found for ICSI it must be a healthy cell capable of achieving fertilization" (Lamb, 2010). Lamb (2010) suggests that some couples might feel empowered by finding the specific causes to their infertility, whether the information helps guide them to a solution, such as a potential treatment like ICSI or other options such as donor or adoption; the information may also lead some couples towards a decision to not have children, if a procedure does not feel right, or it is too financially draining, etc. "Knowledge of the cause of their infertility may provide closure for couples and a sense of confidence regarding their choice or reproductive

treatment” (Lamb, 2010). Moreover, it seems that this type of information must be explored, not only for the end goal of creating families, but because we may stumble upon things that we didn’t even know were related to infertility, and we may find insight into new ways of treatment (Lamb, 2010).

According to Barrat et al., (2011) other specialists have a different perspective: “[to date], the assessment of sperm function has failed to make significant impact on the clinical management of couples.” And the basic reason for this seems to be that “the logarithmic progress that has been made in understanding the basic science around how spermatozoon develops, prepares for fertilization and contributes to a healthy birth has not [yet] been translated into routine clinical practice” (Barrat et al., 2011).

### *Image Cytometry*

Image cytometry is based on the measurement of absorbed light at each point of a sperm cell on a stained smear under a microscope. This approach allows for precise and reproducible measurements of cell structure and function, and relies on image analysis systems that combine microscopy, video and data processing. In order for this system to work, the quality of preparation, choice and quality of fixing, type of preparation, dye, light, and adjustment of optics must be carefully chosen. Although this approach shows promise in determining sperm biometrics in various clinical and research settings, it’s still not known if it will be a good routine test in the approach for analyzing sperm

morphology for fertility purposes. This is especially because of its inability to categorize sperm defects and its inability to perform pattern recognition (Auger, 2010).

These newer, computer-based approaches should be considered complimentary approaches, “rather than [exclusive] or redundant tools for the assessment of multiple aspects of microscopic anatomy of human spermatozoa... a laboratory involved in the diagnosis of male infertility and ART should not only evaluate typical spermatozoa, but also categorize the various sperm defects, allowing for the calculation of a useful teratozoospermia index” (Auger, 2010). Perhaps in the future, a potential male-factor patient will have more choices for testing, and the tests performed will be based on the individual.

### ***Tests to Measure the Quality of Spermatozoa at Spermiation***

One study by Amann (2010) includes a critique of the World health Organization’s (WHO) revised semen analysis manual, especially in relation to spermatozoon at spermiation. The WHO claims that this newer analysis will be helpful for investigating male fertility status and for monitoring spermatogenesis during male fertility regulation. Amann (2010) states that this is basically not possible unless the specific cells are identified and tested. Therefore, the new guidelines may help in gaining somewhat of an understanding of whether the male will be able to impregnate his partner to some extent, but it will give no specific information about the testes, illness in the testes or the specifics as to what is really wrong with the male partner (Amann, 2010). He specifically

questions how to find the difference in measurement in terms of detection of spermatozoa that were abnormal at spermiation vs. those that became abnormal subsequently (Amann, 2010). Amann goes on to state that these “quality” assessments are meaningless, and suggests the future is in finding alternative quality attributes defining spermatozoa at spermiation. In conclusion, there needs to be a new paradigm for the morphological evaluation of sperm quality and new technologies to test several independent attributes and values within a sample and spermatozoon in order to more accurately identify the proportion of abnormal spermatozoa (Amann, 2010).

It is interesting to note that Amann (2010) mentions studying the organs and overall “illness” within an infertile male (a holistic approach congruent with TCM theory) suggesting that a systematic illness may have an effect on sperm quality and male factor fertility. So perhaps blood tests beyond what men are having done today will be helpful in locating where the dysfunction happens in the system and therefore how it is effecting the sperm and fertility of the couple. One team Amann examines is a team of an andrologist and an epidemiologist who are attempting to learn about another factor in relation to infertility and teste health. They are studying Leydig and Sertoli cells and spermatogenesis, an area which seems to hold many potential answers (Amann, 2010).

Another idea presented is whether pathological changes in sperm are from the DNA or from the epididymis. Amann (2010) states that changes could be the result of the interaction of the spermatozoa with successive microenvironments in the lumen of the epididymal duct. Moreover, the next question is whether the failure of a spermatozoon to

undergo normal changes in its transit through the epididymus is due to abnormal spermatogenesis or due to epididymal dysfunction. He also hits on the idea that certain manifestations of dysfunction in the beginning of the process can influence how things develop downstream. This may make the evaluation and finding the pathology and appropriate treatment very difficult (Amann, 2010).

Interestingly Amann (2010) states that “it is important to acknowledge that no one can identify a normal spermatozoon – not today, and probably not 25 years from today.” He discusses how during spermiogenesis, maturation and the mixture of seminal fluids in the laboratory there are over 100 processes that take place and most go right. Or, at least we assume so, although some may not go right, while other processes go smoothly and help compensate for the subfunction at other points in the process. Thus, “one should evaluate multiple independent attributes for each spermatozoon and then identify cells that are definitely abnormal in one or more of those attributes thought to be important in bastula-producing spermatozoa” (Amann, 2010).

He also states that it may be important to start classifying male factor and sperm quality issues into two classes: compensable and “uncompensable” (Amann, 2010). For example, sperm issues may be overcome, especially in relation to sperm motion or sperm-zona binding by insemination or more sperm (which would be compensable), whereas chromosomal issues and issues beginning with DNA defects would be perhaps more difficult to treat and considered uncompensable (Amann, 2010).

Overall he foresees a matrix of continuous or binomial responses for attributes and values expressed in each spermatozoon, “measured for multiple cells within a sub sample, would be preferable to single or several concurrent bulk measures on a population of spermatozoa” (Amann, 2010).

### *Miscellaneous Sperm Testing and Treatment Ideas*

One test that shows promise is a primary functional assay, the human zona—binding test, which may help with ART in the future. Another test that shows promise is a test involving calcium and sperm development, as decades of research have shown that calcium regulation can be used as way to potentially identify dysfunctional cells. However, more robust assays are greatly needed. Currently the author uses a FLUOstar assay (BMG Labtech in Offenburg, Germany), which is efficient and accurate for calcium mobilization detection in semen samples. However, it requires .5 million cells to get the robust data points which most men who want ART do not have, and they are the ones who have the most to gain from this type of assay (Barratt et al., 2011).

Home testing may come shortly. Again, proteomics is increasingly being applied to andrology to find answers. Metabolic profiling of blood samples may also help with identifying subfertility, which may eventually help in the field of ART. Also, the Raman spectroscopy shows promise in identifying genetic issues, which may help in ICSI (Barratt et al., 2011).

Some other testing ideas include testicular retrieval of sperm for ART as opposed to epididymal retrieval, although recent research states location doesn't make a difference (Marchesi & Feng, 2007). Another tool may be high magnification optical systems (6600x as opposed to 400x) for picking out the best option for ICSI (Marchesi & Feng, 2007). Also, another tool may involve lowering centrifugal forces, or simply using swim-up techniques instead to avoid damage. Sperm washing mediums seem to actually be helpful because they may inhibit bacterial binding and diminish nDNA damage caused by ROS (Marchesi & Feng, 2007). In vitro culturing of surgically retrieved testicular sperm for 48-72 hours at 37°C may improve motility (Marchesi & Feng, 2007). Finally, sperm-hyaluronan binding assay (HBA) might be a potential assay for isolating viable sperm, but again, there are many issues that need to be worked out in order for this test to be truly valuable (Marchesi & Feng, 2007).

### **DNA Fragmentation Index (DFI)**

#### ***DNA Fragmentation and the DFI Test***

DNA sperm fragmentation can result in damaged sperm and lead to infertility. There needs to be a greater understanding of the DNA in sperm, and how to test it in relation to fertility. This may be an important part of treating infertility successfully (Marchesi & Feng, 2007).

There are two types of DNA within the sperm: Nuclear DNA (nDNA) & Mitochondrial DNA (mtDNA). nDNA is located in the head of the sperm and contains all of the paternal genetic information that will be needed for fertilization. It is referred to as the genome. One might associate this part of the DNA as “yin” DNA, as it is hidden. mtDNA is located in the midpiece of the sperm, and with the use of the tail, helps in mobilizing the sperm towards the egg for fertilization. This might be considered the “yang” type of DNA, which is active and uses a tail to guide and direct. It provides ATP for cellular acceleration. nDNA can be damaged through defective chromatin packaging, apoptosis, OS and lesions (genetic). mtDNA is damaged mainly through deletion and copy number issues. Scientists are also trying to see their symbiotic relationship, how they affect each other, and how this will effect testing, diagnosis and the endpoint in fertility treatment and ART (Marchesi & Feng 2007).

There are many possible causes of DNA fragmentation, such as abnormal packaging and segregation of chromatin, oxidative stress, and abnormal cell apoptosis (Xu ZP et al., 2008). According to Nicopoullou et al., 2008, there are several theories behind the pathology of DNA fragmentation. The first reason may be the DNA breaks failing to “re-ligate during chromatin packaging” (Nicopoullou et al., 2008). The second cause may be in relation to apoptotic DNA cleavage in germ cells, leaving trapped dying cells in the testis. The third reason may be that fragmentation may occur with maturation and ejaculation, “due to excessive production of reactive oxygen species (leading to abnormal ligation, apoptosis and necrosis) and subsequent cellular damage” (Nicopoullou et al., 2008).

In some studies, semen variables don't seem to correlate with DFI measurements (Nicopoullos et al., 2008). Morphology and DFI show correlations in some studies (Nicopoullos et al., 2008), but larger studies need to be done to really know. For example, if a sample is normal in count and motility, but has slightly less than normal morphology and less than 30% DFI, the couple might be counseled to try naturally or try IUI. If the DFI was greater than 30%, they might be counseled to pursue ART (Nicopoullos et al., 2008; Simon et al., 2010).

It is interesting to note that oocytes are able to repair sperm DNA damage, but this depends on the level of damage and the oocyte's capacity to repair and heal this damage (Nicopoullos, 2008). This suggests that no matter what the issues are with the sperm, maternal age may matter more, as a stronger oocyte can repair sperm even better than a damaged or aged oocyte (Simon et al., 2010).

Recently DNA testing has become more and more recognized as a promising tool for helping couples conceive:

“Measurement of sperm DNA damage is a useful biomarker for infertility with numerous studies showing its association with longer times to conceive compared with fertile couples, impaired embryo cleavage, higher miscarriage rates and also significantly increased risk of pregnancy loss after IVF and ICSI” (Simon et al., 2010).

### ***Types of DFI Tests***

In terms of DFI tests, the SCSA (Sperm Chromatin Structure Assay) and the terminal deoxynucleotidyl-transferase-mediated dUTP nick-end labeling assay (TUNEL), and the Comet assay have been used with some success, all showing a slightly different set of information (Simon et al., 2010). For example, the Comet assay seems to be the most sensitive. It measures damage in individual cells, and in both single and double strand breaks, and is highly reproducible. It also requires such a small amount of cells that it may be very useful in cases of oligozoospermia, or when testicular samples are taken (Simon et al., 2010).

### ***DFI and its Role in Assisted Reproduction: IUI, IVF and ICSI***

Elevated DFI may contribute to a lower pregnancy rate in ART, recurrent spontaneous abortions, and “potential genetic risk of ICSI offspring” (Xu ZP et al., 2008). Some treatment strategies recently explored to lessen fragmentation and increase ART outcomes include what Xu ZP et al., (2008) called “antioxigen drugs”. Likely he might have been mistranslated and the author might have meant “antioxidant therapies”. Other treatment strategies include ICSI with sperm from the testis, using frozen sperm, removing “etiological factors,” which Xu ZP et al. (2008) do not specify, and traditional Chinese medicine (Xu ZP et al., 2008).

One meta-analysis showed that elevated DFI affects in vitro fertilization and IUI, but that DFI was not a good predictor of ICSI success (Nicopoullou, 2008). Nicopoullou, (2008) recommends, “DNA fragmentation be used as a part of the standard evaluation of subfertile men, with the standard semen variables to aid the decision on what treatment is required.” For example, couples could be counseled about the poorer outcomes of IUI and IVF with DFI scores higher than 30%, and therefore ICSI might be a better treatment. Although this idea is contrary to previous ideas that DFI may also be a helpful test when it comes to ICSI (Nicopoullou, 2008).

Pregnancy rates are, statistically, significantly higher for men with a DFI below the thresholds of 30-40%. One study shows that no pregnancy results when the DNA Fragmentation Index is greater than 12% (Evenson & Wixon, 2008). In Sweden, their regional government will not fund an IUI procedure if the patient’s SCSA-defined sperm DNA fragmentation score is greater than 25%. Overall perspective is that “sperm DNA damage is more common in infertile men and may contribute to poor reproductive performance” (Evenson & Wixon, 2008).

One article studied 360 couples and found that couples that failed to achieve a clinical pregnancy had higher levels of DNA fragmentation than the successful couples (Simon et al., 2010). Overall the authors concluded that DNA fragmentation “can predict ART outcome for IVF...[and] converting [modified bases] into further DNA strand breaks

increased the test sensitivity, giving negative correlations between DNA fragmentation and clinical pregnancies for ICSI as well as IVF” (Simon et al., 2010).

The effects DNA damage may have are even farther reaching as DNA damage may not only effect whether a couple conceives and delivers a child, but may also effect the germ line for future generations to come (Simon et al., 2010). Sperm with oxidative stress and DNA damage has shown to have the ability to not only reach the egg, but also successfully fertilize it and implant, possibly causing mutations during embryological development, and possible loss of the fetus. If this damaged DNA becomes “incorporated into the embryonic genome, it may lead to errors in DNA replication, transcription and translation during embryogenesis, contributing to a number of human diseases in not just one, but subsequent generations...in particular, sperm DNA can impact on the short and long term health of children born by ART” (Simon et al., 2010). Some studies show that children born from ICSI in particular have a slightly higher risk of having health issues (Simon et al., 2010). Some studies also are linking DNA damage in sperm to childhood diseases such as cancer and autism, which seems to be exacerbated by paternal smoking (Simon et al., 2010). Currently children from ICSI have about a 10% chance of having a congenital malformation, while those children conceived spontaneously have about a 3% chance. This research is controversial as some reviews say there is little to no difference (Simon et al., 2010).

Although more studies need to be done, there is a rapid accumulation of studies showing that DNA damage affects pregnancy outcome in ART, and that it is higher with ICSI

patients (Simon et al., 2010). This is in contrast to other studies, which suggest that ICSI patients should utilize DNA testing (Simon et al., 2010). Other articles say that DFI tests may be important for IUI and IVF, but once you are at the stage of utilizing ICSI, the bypassing of motility testing etc. allows the couple to use their “best sperm”, not realizing that motility may or may not be related to sperm quality and the health of their potential offspring (Simon et al., 2010).

Another recent study investigated implantation and pregnancy rates with ICSI and IVF, using sperm with high levels of DNA fragmentation (Speyer et al., 2010). It describes the difference between two different instruments. First, the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay, which identifies DNA breaks by labeling 3'OH termini, and is a measure of existing DNA damage. The other instrument is the sperm chromatin structure assay (SCSA), which measures single-stranded DNA after acid treatment, including potential DNA damage (Speyer et al., 2010).

When using intrauterine insemination (IUI), pregnancy and sperm DNA fragmentation Index (DFI) is inversely proportional. The higher the DFI, the lower the rate of pregnancies after IUI (Speyer et al., 2010). When it comes to finding out if a high DFI affects pregnancy rates in IVF and ICSI, the evidence is controversial. A 2006 meta-analysis concluded that elevated DFI affects IVF cycles but not ICSI, and therefore patients with sperm fragmentation should use ICSI, when the fragmentation was measured with the TUNEL assay. According to some past studies, when using the SCSA

assay, DFI didn't seem to have an effect on either IVF or ICSI. This study tried to find out whether high DFI had an overall effect on pregnancy during IVF and ICSI, and if so, where exactly in the process this occurred (Speyer et al., 2010).

One thing to consider in this study is that the ICSI and IVF patients differed significantly in one way: The ICSI patients had a higher rate of male factor infertility, and lower sperm concentrations, motility and morphology. During the cycles, they had lower 2PN embryos, morulas and blastocytes, in comparison with the IVF patients, where there was more female factor. This of course is part of the reason why patients with male factor were advised to do ICSI in the first place (Speyer et al., 2010).

The study by Speyer et al., (2010) helps to resolve issues about how and to what extent DNA fragmentation may impact upon the success of IVF and ICSI (Speyer et al., 2010). Their observations confirm the inverse relationship between DFI and motility, which explains why there may be a difference between IVF and ICSI when it comes to DFI. "The spermatozoa with high DFI may lack certain properties required for IVF of an egg, the most obvious of which is motility, and in IVF cycles they will be less able to compete against any spermatozoa present in the same sample which possess relatively normal characteristics, resulting in a more normal spermatozoon fertilizing the egg. When doing ICSI, embryologists attempt to pick out the best spermatozoa, but some types of damage may not be seen" (Speyer et al., 2010). It seems that in ICSI, motility is not considered, because it doesn't really have to be considered. The RE will insert the sperm into the egg, and motility is not needed in this process, per se. The observation here is that motility is

in fact just as important as other characteristics, and should still be measured in some way before ICSI, as poor motility is related to DNA damage and lower rates of implantation. The solution to this may be finding better ways to pick out the spermatozoa, such as high magnification inspection of morphology (Speyer et al., 2010).

There is a strong correlation between DFI and midpiece defects, especially in ICSI cycles. The defects are possibly a result of disordered spermiogenesis, “leading to enzyme-containing residual cytoplasm in the midpiece”, and may initiate a role in the fragmentation process. Overall, both low motility and midpiece defects in a routine SA may suggest elevated DFI (Speyer et al., 2010).

The conclusion of this study is that >30% DFI is a possible threshold for IVF cycles, and high DFI measurements are strongly correlated with low implantation rates in ICSI and low pregnancy rates in both ICSI and IVF. Also, the DFI value does not negatively affect fertilization or growth of the embryo up to day three (after fertilization). It does, however, affect post blastocyst development and implantation. Again, sperm that has higher than 19% DFI when used in ICSI was associated with lower implantation rates (Speyer et al., 2010). The study also raised the concern that “there may be long-term effects on offspring of using high DFI spermatozoa (...) as long as this remains a possibility every effort should be made to find ways of lowering the DFI, such as by treatment with oral antioxidants” (Speyer et al., 2010).

## **Future Research**

The sperm chromatin DNA damage assays have considerable challenge, despite their seemingly impressive placement in the armamentarium of diagnostic tools since 1980. Despite the controversy, the DFI seems to be able to offer much insight and should still be considered as a possibility of future testing as a part of clinical management (Barratt et al., 2011).

In regards to DNA structure testing via a sperm chromatin structure assay some specialists feel that it may be useless for evaluating quality of sperm leaving the seminiferous epithelium. Amann (2010) states that it may be more appropriate for certain tests, including the DNA structure test, to study sperm from the efferent ducts or proximal epididymis and from the cauda epididymus or vas deferens, using tissue from consenting organ donors who have passed unexpectedly. He goes on to state “future analyses of individual spermatozoa within populations from the proximal and distal epididymal duct might allow a better classification of these tests as appropriate or inappropriate for evaluating the quality of spermatozoa at spermiation” (Amann, 2010).

## **Acupuncture and Possible Effect on Sperm Quality and DFI**

One of the main modalities of TCM, acupuncture, has shown to be effective in treating several health issues including infertility. The WHO states that acupuncture can effectively treat pain, depression, digestive complaints and hormonal imbalances. The

mechanisms behind acupuncture are still being explored. The main theory is that acupuncture has a neuromodulatory effect. Needling an acupuncture point stimulates the nervous system to release chemicals in the muscles, spinal cord, and brain. These chemicals will either change the experience of pain, or they will trigger the release of other chemicals and hormones which influence the body's own internal regulating system. The improved energy and biochemical balance produced by acupuncture results in stimulating the body's natural healing abilities, and in promoting physical and emotional well-being. This ability to affect neuromodulation may be one of the reasons why acupuncture may be helpful in decreasing DFI. Acupuncture has been shown to successfully treat poor sperm density, especially when in conjunction with inflammation of the genital tract (Pie et al., 2005). General improvement of sperm quality after acupuncture treatment has been suggested in several studies, specifically, of the ultrastructural integrity of spermatozoa. A more in depth review of this portion of the literature will be discussed in detail in the Results and Discussion section (Agarwal et al., 2003; Pei et al., 2005; Sherman et al., 1997; Ward, 2009; Zhang et al., 2002).

### **Herbal Medicine and Possible Effect on Sperm Quality and DFI**

Another component of TCM is herbal medicine. Traditional Chinese herbal medicine has been used for thousands of years to treat a variety of health conditions, including reproductive imbalances both for men and women as well as pre-conception care even in ancient Chinese culture. It is proven effective in treating pain, digestive disorders, infection, anemia, anxiety and a number of other health concerns. The antioxidants

contained in the herbs may be one reason why certain herbs are being prescribed. Many formulas increase circulation, reduce inflammation and calm the nervous system. All of these components may have an effect on sperm DNA fragmentation. Moreover, growing evidence suggests that seminal oxidative stress is involved in many cases of idiopathic male factor infertility. As antioxidants may decrease the oxidative process, herbal formulas, some of which contain antioxidants, may help to reduce oxidation and improve male factor fertility, and possibly prevent sperm fragmentation (Tempest et al., 2008; Kaleem et al., 2009; Lyttleton 2004).

Dang Gui is an herb that is thought to have an effect on estrogen, although more studies need to be done (Damone, 2008). Many herbs are thought to have androgenic and estrogenic effects. Herbs such as Ba Ji Tian, She Chuang Zi, Suo Yang, Tu Si Si and Yin Yang Huo (epimedium) increase the secretion of endogenous hormones such as corticosterone, cortisol, and testosterone (Chen, 2001). All of these botanicals may have an effect on sperm DNA fragmentation, sperm quality and improving male infertility. Herbal formulations that contain antioxidants and support hormonal function may help to reduce oxidative stress and improve male factor fertility by possibly reducing sperm fragmentation (Tempest et al., 2008; Kaleem et al., 2009). A more in depth review of this aspect of the literature will be discussed in detail in the Results and Discussion section.

## **Literature Review Integration**

Prior research has given possible explanations as to why male infertility is on the rise. Studies have been done on what the best ways are to test sperm quality, both in terms of traditional testing such as the semen analysis, and modern testing such as the DFI test, and how testing of semen gives insight into possible treatments for male infertility.

There have been some studies on TCM modalities affecting semen quality and male infertility. Both acupuncture and herbs have been used to test their potential effect on the traditional semen analysis, changes in hormone levels, antioxidative levels, quality and quantity of immune markers, fertilization rates, pregnancy rates, and in a few cases, live birth rates. What has not been done is a complete literature synthesis on the topic of TCM (both acupuncture and herbal medicine) and its possible effect on DFI, semen analysis and male infertility. This study attempts to contribute to the investigation of TCM's potential to help improve male infertility, while taking into consideration the newest ways to test sperm quality and measure male infertility or subfertility. Specifically this is an attempt to investigate possible effects of TCM on DFI, and overall male infertility.

## **Chapter 3: Methodology**

### **Objective**

This literature synthesis will be used to explore sperm quality, in relation to male factor infertility, and possibilities of treatment; specifically, Traditional Chinese Medicine (TCM) and its possible effect on sperm DNA fragmentation Index (DFI). This topic will be investigated to discover if there has been any evidence that acupuncture and Chinese herbal medicine has an effect on DFI levels, sperm quality or male infertility. My hypothesis is that TCM increases semen quality, lowers DNA fragmentation and increases the change of pregnancy outcome in couples who have been diagnosed with male factor infertility.

### **General Statement of Methodology**

The literature review will consist of reviewing articles that are relevant to the topic of Traditional Chinese Medicine and treatment of poor sperm quality and DNA fragmentation. The increase of male factor infertility will be explored, followed by taking a look at traditional ways of measuring sperm quality such as the traditional semen analysis. Then some of the more current and experimental ways of measuring semen quality, such as the DNA fragmentation index (DFI) will be looked at. Articles will also be sought to explore DFI and its relation with sperm quality and embryo quality. Articles will be included on what causes sperm DNA fragmentation, as well as TCM's modalities

and their relationship with the etiologies of DNA fragmentation. Acupuncture and its effect on sperm quality will be explored, as well as the possible herbal effects on sperm quality, male infertility and pregnancy rates, if available.

The literature synthesis method was chosen to find answers to the hypothesis that TCM has a positive effect on DFI. I am specifically interested in studying TCM and DFI because I will potentially be doing research with male infertility subjects. Thus I needed to know what had been done already before pursuing research on the topic.

### **Instruments and Procedures**

To find articles and studies related to the research question, online search engines such as Google Scholar and PubMed will be utilized. Fertility & Sterility, Human Reproduction and several other peer-reviewed medical journals will be used to find related articles and studies pertaining to the research question. I will utilize the University of California system through UCLA medical library as well.

For searches, key words and phrases will include: Traditional Chinese Medicine (TCM) effect on Sperm Quality, DNA Fragmentation Index (DFI), TCM effect on sperm quality, male infertility, In Vitro Fertilization (IVF), Assisted Reproductive Technologies (ART), Etiology of DNA Fragmentation, Herbal Medicine effect on Sperm Quality, Acupuncture effect on sperm quality.

This part of the Methodology Chapter will describe the procedures and methods of Data Analysis, and how I will organize, display and report my results and data as they are extracted from the data collection forms devised for the review of each article used in the Literature Review.

### **Reports and Displays: Tables**

The primary step of organizing, displaying and reporting the data will be to organize them into table form. The tables (Table 1-4) will list the article's name, author and date written on the left-hand margin, and the top column will show representing themes or concepts respectively. The boxes in the center will have an "x" or a blank field, representing whether or not the article contains any information pertaining to the theme indicated above. This display and report is to help the researcher organize the information and articles on a deeper level.

### **Inclusion and Exclusion Criteria**

#### ***Inclusion Criteria***

Of 35 articles in Chinese language found, only six studies were included in this analysis. I chose to include some of the studies published in Chinese language as there were only few studies done on herbal medicine in the English language. Also, it seemed that the Chinese language articles might help to give some idea as to what has been done, even if

the studies are not exactly at the same level as English language studies. As there is so little information, it appeared helpful to gain a better understanding of the concept. Part of the reason for excluding most Chinese studies was the language barrier and a lack of translational work, lack of funding to purchase large amounts of translated work, and the inability to grade a study's quality as China has different ways of running studies. It also seems the studies from China show a trend in that not all of the herbs were disclosed in the formula used. Some studies listed a few herbs and then continued with "etc." to indicate that there were more herbs, but they would not list them (Zhiyuan, 1996).

Although including reviews is discouraged in current Literature Syntheses for the most part, review articles were included because these reviews gave important information and therefore potential insight that would have been overlooked otherwise. The other reason for including reviews is the general the lack of studies in this area. At this juncture, specializing scientists, Urologists, Andrologists, OBGYN's, RE's, Immunologists, Integrative fertility specialists and, most of all, concerned patients need all the help they can get. Thus, including all pertinent information in this analysis may eventually lead to more efficient, less costly and overall better care, and is of necessity. For example one article consisting of a review of case studies showed very high pregnancy rates (55.6%), after only three courses of treatment (approximately 30 TCM treatments). This case review study suggests that acupuncture may be effective in improving chances of pregnancy in male infertility with a minimum of 30 TCM treatments required (Zhiyuan, 1996). Another study within a review reported up to a 78% pregnancy rate in available couples. All subjects had received a Chinese herbal remedy twice a day for 60 days

(Crimmel et al., 2001). Both of these studies were in reviews, and show that TCM was efficacious in safely treating male infertility.

### ***Exclusion Criteria***

Studies before 1990 were not included. Animal studies were not included. Studies revolved around the investigation of nutritional supplements or solely antioxidants and sperm quality were not included, as the main research question addressed in this study was related to TCM in its most holistic form, using acupuncture and herbal therapeutics. Although, there may be significant antioxidant activity in some of the herbs and formulas used in the analyzed studies, this was not the focus of the study. This should be investigated in a future study.

### **Validity and Reliability**

Perceived threats to validity and reliability are related to the forms used and the peer-reviewed journals accessed. One remaining threat to validity and reliability is related to the fact that this study is somewhat limited as certain articles that may be helpful for the review were not accessible, which may affect the validity of the review/synthesis. The overall effect should be minimal, as many of the studies were repeated and written about in more than one journal, so the majority of the main concepts will be covered and discussed.

Data are gathered from each reviewed study in a systematic manner using the data collection forms (see appendices). The use of the forms increases the reliability of the data. In terms of the actual journal articles, articles were collected from published sources and peer-reviewed journals. These publications usually share standards for acceptance of articles and studies that adhere to accepted research practices within the profession. This factor increases the reliability and validity of the data collected from each of the articles reviewed and used for the synthesis. Internal threats may include the possibility of incorrectly summarizing an article, but this should be minimal due to training in research methodology and near completion of three doctoral level courses in research methodology training in the doctoral program at Yo San University.

### **Methods Used to Analyze Data**

Thematic content analysis is the qualitative method used in this literature synthesis. It is an appropriate method often used for review and synthesis of published research because the framework is more qualitative and broad. Therefore data need to be reviewed and then grouped into sections in order to convey the summaries and allow for more accurate and organized analysis (Roberts, 2008).

### **Rationale**

Thematic content analysis is used for the purpose of completing this study which may lead to the elucidation of factors that may be specified for further exploration in future

systematic studies. The qualitative method is appropriate for novel and timely topics where there has not been a lot of prior research. The qualitative method is more effective to discern the similarities and differences, different themes and patterns, and other qualitative phenomena. This method is more open and limitless, thus allowing the opportunity to identify factors that may be significant, and perhaps overlooked in the past, especially if investigated through a strictly quantitative study (Roberts, 2008).

## **Chapter Four: Results**

### **Data Overview**

Data were gathered via Google scholar and through the UC system using key words pertaining to the topics, including semen quality, male infertility, alternative treatments for male fertility and DFI.

### **TCM Treatment Modalities for Male Infertility**

Of the 27 articles gathered on TCM and its effects on semen quality, DFI and male factor infertility, there were two main TCM treatment modalities used to test a possible effect (acupuncture and Chinese herbal medicine). Three of the acupuncture studies included moxibustion. Two of which actually used both modalities in the study and one of which included both in their review. One study used a combination approach using both acupuncture and herbs. Nine of the herbal studies used herbs via internal consumption (in vivo). Four studies used the herbs in vitro, by actually bathing the semen in an herbal solution. In summary, twelve articles looked at acupuncture, thirteen looked at herbs, one looked at both acupuncture and herbs within a review of cases, and one study used a combination of both acupuncture and herbal therapy. In the combination study, investigators used a combined therapeutic approach of both acupuncture and Chinese herbal medicine to treat specifically immune-related male infertility.

## **Methods of Conception**

Studies analyzed reported the use of both Artificial Reproductive Technologies (ART), such as IVF or ICSI, as well as Natural Conception Methods (NCM) in an attempt to achieve a pregnancy. Some studies did not mention or measure the method of conception, as this may not have been included in all of the studies. Of the 27 articles, four noted that the method used was ART and four noted that the subjects used natural conception methods.

Of the studies examining ART, there was a trend for increase in fertilization rate after acupuncture therapy. In a prospective controlled pilot study (Siterman et al., 2000) 40 men received acupuncture twice a week for five weeks, receiving their own customized acupuncture treatment. Subjects had low sperm count, azoospermia, or oligospermia. Thirteen of 20 had an increase in sperm density, 67% had an increase in count, and two couples underwent ICSI and both conceived (Siterman et al., 2000). One acupuncture study (Zhang et al., 2002) had an increase in form and motility, but not concentration with two acupuncture treatments per week for eight weeks. The 20 subjects had a history of idiopathic male infertility with one failed ICSI. Fertilization improved from 40.2% to 66.2% (Zhang et al., 2002). One review (Huang & Chen, 2008) reported that herbal formulas created a balance in hormones, an increase in fertilization and pregnancy rates, and a decrease in immune markers. Specifically, Huang and Chen (2008) included a study by Lian et al. which used a combination therapy of Zhuanyindan and prednisone to

treat male infertility with AsAb, and reported improvement in sperm quality by decreasing the nitric oxide level in semen. One ART study concluded that there were not enough randomized studies; however, pregnancy rate of IVF was higher when acupuncture was given to the female on the day of the embryo transfer compared to when subjects had an embryo transfer without acupuncture.

In the cases of natural conception methods, the articles stated that there were increases in pregnancy rates with the use of acupuncture or herbal medicine. In one article (Xu et al., 2003) the authors discuss a study using Shengjing pill with 202 subjects for two months. Subjects had been diagnosed with semen abnormalities. The concentrations of serum FSH, LH and testosterone were normalized by treatment, and 78% of the 116 spouses conceived (Chen and Wen 1995). “In addition, two observation studies confirmed the improvement in serum FSH, LH testosterone, corticosterone and pregnancy rate after herbal treatment, indicating that the herbs had multiple actions on the pituitary axis (Yue et al., 1996; Yang et al., 2001)” (Xu et al., 2003). These studies show that TCM can be helpful in the treatment of male infertility and improving sperm quality whether the method of conception is natural or assisted.

### **Measurements Used to Assess Effect of TCM on Male Infertility**

To see how TCM may be affecting Male Infertility in the analyzed studies certain measurements were tracked, such as semen analysis, hormone or immune markers, antioxidants, as well as fertilization, pregnancy and live birth rates. Interestingly, the

most important outcome measure, the birth of a healthy child, was not measured in most of the articles or studies. Only six of the 27 articles tracked this outcome measurement. Deadman (2008) mentioned the measurement of DFI but none of the actual studies used this as a measurement.

In terms of measuring semen quality, one investigator and his colleagues created their own “Fertility Index”. Overall many of the articles used different measurements within a traditional SA, for example, measuring ejaculatory or erectile dysfunction, +plasma (aFas), disomy (an extra chromosome), acrosin levels, sperm capillary penetration and hypo-osmotic swelling, along with the traditional measurements of count, volume, concentration, motility, morphology and figure or head shape.

The hormones measured included:

Prolactin (PRL)

Testosterone (T)

Follicle Stimulating Hormone (FSH)

Luteinizing Hormone (LH)

Hormones levels also included corticosterone and various endocrine assays. TCM’s potential effect on SA and hormones will be discussed in the following sub-chapters in the Acupuncture and Herbal Medicine results section.

In studies measuring antioxidants, blood serum and oxidative biomarkers, antioxidant assays were used as well to find what antioxidative activity occurred in certain Chinese herbs. Some of the herbal formulas stuck to measuring the anti-oxidative effects, either within the plant itself, or from measuring blood serum levels in subjects after treatment. Others used AsAb to measure an effect. Some of the acupuncture studies included the investigation of additional issues, such as vericocele, prostatitis or infection, erectile or ejaculatory function, or issues within the context of ART.

### **Results of the Use of Acupuncture Therapy to Treat Male Infertility**

Many study designs were used in this literature synthesis: prospective, (non)randomized, controlled, single blind, case studies and reviews, either of one single case or a systematic review looking at several studies.

Reviews were included because it was assumed that additional insight would be helpful. For example, one of the reviews reported a very high rate of pregnancies after 30 acupuncture treatments, suggesting that acupuncture may be effective in improving chances of pregnancy in male infertility, but with a minimum of 30 treatments (Zhiyuan, 1996).

Number of men studied (n), duration of the treatment (both minutes with retained needles as well as amount of treatments), number of points used, which points used, measurement of semen quality (both before and after), and finally, pregnancy rates were all examined.

Age was not specifically included as the age range was about 30-45 years of age which was fairly consistent in all studies, and typical for infertile or sub-fertile men seeking treatment. The number of subjects used in the analyzed studies is small, ranging from one, used in a single case study, to 100.

The number of acupuncture treatments given seemed to average twice weekly. The more frequent the acupuncture, the more increase there was in semen quality, especially in one case study which used acupuncture therapy for 10 treatments, given in 2-3 day intervals. This patient's sperm count went from 0.54 to 46.5 million. His sperm density increased from 0.2 million/mL to 18.6 million/mL (Clicci, 2008).

Of the acupuncture points used, the abdominal points dominated (see Table 3.) which directly increase blood-flow to the lower abdominal cavity, and potentially affect circulation in the reproductive tracts and testis. In terms of location and meridian, Kidney meridian points were included in almost every treatment. From a traditional sense, the Kidney meridian points and Yang tonifying points were predominantly used, such as Kidney 3 and 6. The Kidney and Yang tonifying points on the UB (Urinary Bladder) meridian were also commonly used. Spleen 6 and Stomach 36 were also commonly used, in eight of the nine studies that reported points. In all of these articles, some improvement was shown in semen parameters except in one case (Siterman et al., 2009). In this study, 39 subjects were given eight to ten treatments at a rate of twice per week. No more than 12 points were used at a time. Subjects had low sperm count, elevated scrotal temperature and high levels of gonadotropins, or mixed etiology. There was no improvement in the

subjects who had varicocele, although, there was an increase in integrity overall. Scrotal temperature decreased, and sperm concentration increased. When both inflammation and high FSH was present outcomes were mixed, with mixed results (improvements in semen parameters). Pregnancy rate was not measured (Siterman et al., 2009).

Damp-heat points were also used to clear any excess phlegm or mucus in the system, infection or prostatitis. Some points were used to clear damp heat from a specific location; for example, SP9, LV5, LI11, ST28 and GB41 were used in some studies to treat inflammation in the genital tract. Damp-heat is also sometimes correlated with inflammation. In this study there was an increase in sperm count (67%) and 13 of 20 men had an increase in sperm density. The two couples that proceeded to undergo ICSI both became pregnant (Siterman et al., 2000).

One case review involved 54 subjects using only six acupuncture needles, with additional moxibustion on SP 6 (Spleen six). The men in this group had been diagnosed with abnormal semen parameters and ejaculatory dysfunction. The results showed that after an average of 20.5 30-minute long treatments, 30 of the 54 subjects became pregnant with their partner (55.6%) (Zhiyuan, 1996).

In terms of semen quality, the analyzed studies indicated that there is overall improvement in male infertility when acupuncture therapy is utilized, no matter which aspect is in a state of dysfunction (the immune system, hormonal deficiencies, issues with sperm: oligospermia or insufficient sperm, azoospermia or absence of sperm,

asthenospermia or motility, or teratozoospermia or poor morphology as well as the conception and development/birth process). Also, in one case (Hung Yu Ng et al., 2008) there was an increase in sperm concentration with placebo acupuncture and not with traditional acupuncture.

Men with overall abnormal semen parameters were reported to have an increase in sperm count, motility and pregnancy rate, with 30 acupuncture treatments (Zhiyuan, 1996). In one study (Siterman et al, 1997), sperm count went from  $8.5 \times 10^6/\text{mL}$  to  $19.3 \times 10^6/\text{mL}$  with five weeks of acupuncture treatment. In another study (Siterman et al., 2000) count increased by 67% with only five weeks of treatment. In that same study, two of the couples underwent ICSI treatments, and both conceived, signaling to all fertility specialists that acupuncture treatment twice a week for five weeks may not only help sperm count, but may have other positive effects on the sperm, possibly increasing chances of conception when used in conjunction with ART (Siterman et al., 2000).

Zhang et al. (2002) reported no improvement of sperm concentration. All subjects had been diagnosed with idiopathic male infertility with one failed ICSI. In this study form and motility and improved. More importantly, fertilization improved from 40.2% to 66.2%, raising the question why research and medical practice are not focusing on a more integrative approach, especially as form and possibly motility are just as important when using ART, as the more motile sperm may be stronger or healthier, and form or morphology are important in forming a viable embryo.

One study done in 2005 (Pie et al, 2005) was one of the shortest in treatment course length (five weeks), yet showed a marked improvement in sperm morphology and motility. In a study of case reports done by Crimmel (2001), some men got treated for up to five months, and the results varied from “not reported” to 74% of the couples conceiving or having normalization of semen parameters, suggesting that, overall, length of courses of treatment and frequency may make a difference.

Time being diagnosed as “infertile” may prove significant, as shown by Gurfinkel et al., 2003. The authors worked with men who had not been able to conceive for 11 years or more, and who had semen abnormalities in concentration, morphology and motility. There was a significant increase in “normal form sperm”; however, volume, concentration, progressive motility and number of round cells did not show significant changes. Again, form improved but overall numbers did not. In this study there was no measurement of pregnancy, but one might assume that there may not have been any, which perhaps indicates that the longer a couple waits, the harder time they will have conceiving. Paternal age may be just as important as maternal age, giving rise to a not so common diagnosis: Advanced Paternal Age (APA).

One case study done by Cliaci (2008) showed a subject with a sperm count that started at 0.54 million and went to 46.5 million; density increased from 0.2 million/mL to 18.6 million sperm/mL, a 90-fold increase. Ten acupuncture treatments were given on the Kidney meridian. Cliaci’s next study in 2009 involved 45 subjects, whose semen parameters all increased with acupuncture therapy (about 15 treatments) and the

pregnancy rate increased by about 26%, with 12 births out of 45 couples trying to conceive. Cliaci has shown that count, density and overall semen parameters can improve with acupuncture therapy, especially in conjunction with the most important measurement, live birth (Cliaci, 2008).

### **Results of the Use of Combined Acupuncture and Herbal Medicine to Treat Male Infertility**

Fu (2005) studied immune-infertility, utilizing both acupuncture and herbal therapy in conjunction versus oral prednisone. The study reported a significant decrease in AsAb. Moreover, the effectiveness rate to create pregnancy was 90% when both acupuncture and herbs were used. When oral prednisone was used effectiveness rate was 64%. Unfortunately, live birth rate was not measured.

### **Results of the Use of Herbal Medicine to Treat Male Infertility**

Although there were more studies available on the use of traditional Chinese herbal medicine to treat male infertility than acupuncture, many of them were done in China, and therefore written in Chinese and difficult to assess. Taking a basic look at what is available on the topic, the conclusion is that there seemed to be an increase in sperm quality, and sometimes pregnancies or live births, in many cases when measured.

More specifically, the 2001 (Crimmel et al., 2001) review of acupuncture and herbal medicine in the treatment of male infertility and erectile dysfunction included studies from the 1990's, but may still hold some interesting insight. For example, one study reviewed was by Chen and Wen (1996). They examined the use of Sheng Jing on 202 infertile men. Sheng Jing includes the following herbs:

Lu Jiao Jiao

Yin Yang Huo

Xian Ma

Jun Ying Zi

Wu Wei Zi

Sang Ji Sheng

Shu Di Huang

Gou Qi Zi

Tu Si Zi

Shi Hu

Ba Ji Tian

Fu Pen Zi

Huai Niu Xi

San Leng

E Zhu

The formula was given for 60 days, and showed a significant improvement in density, motility and grade, levels of FSH, LH and T, and a reduction in serum AsAb titers. There was a 78% pregnancy rate reported in the 148 couples available for follow-up, which seems fairly high in comparison to newer studies.

One plant study (Tempest et al., 2008) measured the endocrinological aspects of certain Chinese herbs and found that some herbs had increased estrogenic activity while others showed a decrease. Of the 37 herbs tested, none had androgenic properties, 20 showed strong and 10 weak anti-estrogenic activity. Estrogenic responses were elicited for two herbs, while ten herbs exhibited anti-androgenic responses. In terms of antioxidants three herbs showed strong antioxidant activity, three herbs were described with intermediate antioxidant activity, and one herb's antioxidant activity was reported as weak. Some herbs were found to have strong hydroxyl scavenging activity. The investigator mentioned that one herb in particular (*Radix moridae*) was able to increase production of testosterone and had shown a protective effect against hydrogen peroxide-induced stress in past studies (Chang et al., 2008), but that in their own study it did not show this effect. Tempest et al. stated that they used a dosage 5-fold lower than in the study mentioned, bringing up the question of dosage. Tempest et al., also discusses the herb *Semen cuscutae*, which produced markedly improved motility and stabilized sperm membrane function, and had strong antioxidant activity while exhibiting strong anti-estrogenic activity but no measureable androgenic activity (Tempest et al., 2008).

### **In Vitro Herbal Studies**

Four articles were in vitro studies, two of which were reviews that included an in vitro studies (Crimmel et al., 2001; Xu et al., 2003). In the review by Crimmel (2001) the study conducted by Hong et al. (1992) evaluated the effects of 18 herbal extracts on the in vitro motility of spermatozoa. One herb, *Astragalus membranaceus* (Huang Qi) showed a 1.4-fold increase in motility – quite a promising result, especially for such an unexpected tactic.

Liu et al. (2004) did a study that also looked at herbs used in vitro to treat male infertility and found that Huang Qi and Ci Wu Jia together increased motility. This provides additional proof that Huang Qi holds some promise in treating male factor via in vitro methods.

The other two studies that investigated herbal medicine given in vitro showed an increase in sperm motility. Among them was a study by Jeng et al. (1997) that reported up to 42.8% increase in sperm motility. Amano (1996) showed an increase in seminal plasma (sFas) levels, which correlate with concentration.

Finally, Xian Xu did a systematic review which included in vitro studies, finding that, again, Huang Qi improves sperm quality, along with Xian Mao, Tu Si Zi and Bai Ji.

## **In Vivo Herbal Studies**

Twelve articles did in vivo studies using Chinese herbal medicine, the most common way to treat with herbal medicine. Twelve of the 14 herbal medicine studies involved in vivo herbal medicine therapy.

### ***Formulas Used for Treating Male Infertility***

In terms of in vivo Chinese herbal medicine, several different formulas were used:

Ju Jing powder

Hochu-ekki-to (aka Bu Zhong Yi Qi Tang)

Shao Fu Zhu Yu Tang

Sheng Jing pill

Gui Zhi Fu Ling Wan

Liu Wei Di Huang Wang

Yikang Tang

Zhuanyindan

Tianxiong powder

Wuzi Yanzong pill

Motility, count, density and morphology improved with these formulas, in varying degrees. Shao Fu Zhu Yu Tang showed a specific decrease in free radicals. Tempest et

al. (2005) showed a significant reduction in disomy, and included a large number of herbs and combinations, seemingly treating constitutionally. One study (Sun et al., 2006) showed that Chinese Herbal Medicine can reduce AsAb, agglutination, as well as improve SA and pregnancy rate.

The only study found in which acupuncture and herbs were used in a combined treatment (Fu et al., 2005) showed that 90% of the men treated with herbs and acupuncture achieved a pregnancy with their female partner. These subjects were described as men with immune infertility. The prednisone group achieved 64% effectiveness, suggesting that acupuncture and Chinese herbal medicine, when used together, may prove significantly more effective than simply using acupuncture alone, or pharmaceutical treatment.

Perhaps the most interesting formula study was a study on sperm disomy (Tempest et al., 2005). The authors used FISH (Fluorescence in situ hybridization) on the sperm heads of six men before and during constitutional-based treatment with traditional Chinese herbal medicine to see the effects of the herbs on sperm disomy. There was a significant reduction in disomy.

Xu et al. (2003) also conducted an *in vivo* study and found that Gui Zhi Fu Ling Wan improved semen profile, and that Chinese herbal medicine not only increased sperm density and motility, but modulated circulatory disorders in men with varicocele as well.

### ***Single Herbs Used for Treating Male Infertility***

One single herb studied, Shan Zhu Yu (Jeng et al., 1997), showed an improvement in motility of 42.8%. This herb was basically an isolated substance of *Cornus officinalis*. Ahmad et al. (2010) measured hormonal activity in the herb *Withania somnifera* and found that this herb inhibited lipid peroxidation and protein carbonyl content, along with improving the SA. This study also showed an increase in antioxidant enzymes, a corrective effect on fructose, and an increased serum T and LH, with reduced FSH and PRL, congruent with good semen quality.

### ***Plant Assays Used for Treating Male Infertility***

One study (Tempest et al., 2008), which was a plant assay study, takes herbs commonly used in the practice of TCM and examines their biochemical activity and how they may affect male factor infertility. It investigates single herbs as well as decoctions (herbal formulas which contain several herbs). The focus was on whether or not they had an effect on endocrine activity, as well as on anti-oxidative activity. The journal article also gave some background as to how TCM has been tested throughout the decade and has been found to have “anti-thrombotic, anti-inflammatory, anti-allergic, anti-tussive and anti-bacterial effects [Gong and Sucher 1999].” Overall the study confirms previous reports pertaining to the antioxidant activity of certain herbs (Huang Bai, Nu Zhen Zi, Shan Yao, Shan Zhu Yu, Tu Si Zi, Ze Xie, Ba Ji Tian, Fu Pen Zi, Hong Hua, Cang Zhu, Huang Qi and Jin Yin Hua.) It discusses which herbs have estrogenic/androgenic (or anti-

estrogenic and anti-androgenic) effects. The authors also find that some herbs have a strong hydroxyl scavenging activity, while others are capable of scavenging superoxide radicals, while some inhibit lipid peroxidation (Tempest et al., 2008).

## Chapter 5: Discussion

### Summary of Findings

This Literature Synthesis aimed to investigate TCM's effect on semen parameters and male infertility, focusing on DFI. My hypothesis was that TCM increased semen quality, increased male fertility and lowered DNA fragmentation. No studies were found on using TCM to treat DNA fragmentation. However, studies and case reviews were found that showed an overall improvement in semen quality, male infertility and in some cases pregnancy rate.

No matter which arm of TCM treatment modalities was being used (Acupuncture therapy or Chinese herbal medicine, or both) there seemed to be some improvement in one or more aspects of semen quality and male infertility. One systematic review in 2008 (Huang & Chen, 2008) found that there were not enough randomized studies, and sample sizes were too small to make an overall conclusion about TCM effecting male infertility, but the pregnancy rate from IVF was higher when acupuncture was given the day of embryo transfer (Ng et al., 2008). Overall there was a significant improvement in SA results, hormones levels, immune system markers and live birth rates (Deadman, 2008; Sherman et al, 1997; Crimmel et al, 2001; Fu et al, 2005; Huang & Chen, 2008).

One might hypothesize that TCM may very well have a positive effect on the DFI measurement since it has a positive effect on SA and other male-fertility related

measurements, but to date this has not been investigated. As low DNA fragmentation is associated with better embryo quality, better ART outcomes and higher live birth rates, and since TCM is positively affecting these outcomes, perhaps DNA fragmentation is part of the mechanism creating this improvement (Agarwal et al., 2003; Deadman, 2008; Ward et al., 2009).

However there were not any studies found that actually used DFI as a measurement after testing potential effects of acupuncture or herbs or their combination on semen quality, leaving a hole in current research, and suggesting the need for future investigation. Doubtlessly, more rigorous, high-quality studies are needed that include a higher number of subjects. Nevertheless, there is enough evidence to suggest that TCM improves semen quality and that TCM may very well have a positive effect on DFI, but again, at this point no studies have been done (Deadman, 2008).

### **Using Acupuncture to Treat Male Infertility**

Acupuncture seems to be greatly beneficial in conjunction with ART, especially as it is not introducing yet another chemical into the equation. Acupuncture therapy seems to have an overall positive benefit on both male and female infertility subjects, such as reduction of stress, creating a sense of well-being, and potentially having a positive, homeostatic effect on circulation, hormones, neurotransmitters and the immune system. All of the above factors seem to play a part in both male and female factor, for

preconception care, during both IVF or ICSI protocols as well as procedures such as Paulus's study on acupuncture before and after embryo transfer (Deadman, 2008).

The number of acupuncture treatments given seemed to average twice weekly, a sign that many Acupuncturists may want to have a specific treatment plan meeting this level of treatment for infertile patients seeking care. From personal observation, many L.Ac.'s in the field trend with a once-a-week treatment plan, as this is typically convenient for both patient and Acupuncturist, and tends to be the norm for overall "healthy" individuals with only a single diagnosis. In certain cases, such as immune-related infertility, twice-a-week treatments may serve as particularly helpful as this tactic may be aiding in a quicker recovery as seen in Sherman and Sitermans's various studies involving subjects with elevated WBC counts. This is especially so if a subclinical infection is present, affecting the subject with or without their knowledge (Siterman et al., 2009).

In terms of location and meridian, the Kidney meridian points were included in almost every treatment. From a traditional sense, the Kidney meridian points and Yang-tonifying points are predominantly used, and this makes sense as the Chinese "Kidney" is closely associated with creative energy, both artistically productive and literally reproductive. Anatomically speaking, the Kidney energy may also be related to both the reproductive and hormone systems, possibly affecting the hypothalamic-pituitary-gonadal (HPG) axis. Kidney points may also be an effective choice as they can increase both the "Yin" and "Yang" of Kidney Qi, or Essence. Some points may have more Yin or, in western terms, anti-inflammatory effects, which can be cooling and nourishing, and may have some sort

of strengthening effect in this sense. Other points may have a more Yang effect. Relating to the Chinese idea of increasing robustness, Yang is the warming, activating “male” energy needed for fortitude and movement (or motility) of healthy sperm, in the traditional sense. Kidney Yang deficiency may also be associated with spermatogenic failure (Xu et al., 2003).

The acupuncture point LI11 (Large Intestine 11 or “Pool at the Crook”) was frequently used. LI11 is a point which “clears heat” and “cools the blood” which may be, again, associated with inflammation. This point is located in the area that is, in many Asian cultures, needled in patients presenting with a cold or flu, an acute infection, a systematic or local inflammation (especially expressing heat), an overall immune imbalance, or underlying immune deficiency. For example, in the Japanese “Kiiko” (Kiiko Matsumoto) style of acupuncture therapy, the area near LI10 - 11 is palpated and tested to see if it shifts immune-related symptoms while the patient is literally lying on the table. The entire 2-inch circumference is considered a possible treatment site. This treatment area seems to be consistently used for immune system imbalances in many cultures, bolstering its curiosity as an acupuncture treatment area used for possible treatment of immune-related infertility or prostatitis.

Overall the acupuncture treatments affect different aspects of the sperm in different studies, calling for more studies with a higher amount of subjects and more specificity in regards to subject’s medical history, subject inclusion, type of treatment, outcome measure, etc. One study (Dieterle et al., 2009) also comments on this idea. In their study,

some improvements in sperm quality were made, but they felt that the improvement may not be significant unless there was a higher number of subjects.

Also one study reported an increase in sperm concentration with placebo acupuncture in comparison with traditional acupuncture. This needs to be explored further. If the placebo acupuncture was based on a more *ashi* technique, i.e., a more palpation-based technique, this would lead us to the specific insight that places of tenderness or stagnation may be the key to location and needling successfully. If placebo acupuncture was used in the sense that no needle was inserted, but the patient had a 20 minute consult that involved a full intake, then this would provide insight as well. More information is needed, but what is most important is that there was some improvement in both small and larger studies.

### **Using Chinese Herbal Medicine to Treat Male Infertility**

Not only did the herbs show significant improvement in semen quality when used as a single herb, but it would seem that regardless of whether investigators were testing single herbs or formulas, a significant positive change was shown. This suggests that *in vivo* herbal therapy may hold some role in future treatment of male infertility. Moreover, the use of many herbs together in an herbal formula, treating constitutionally according to the patient's individual TCM diagnosis, and treating sperm *in vitro* with certain herbs also proved promising to improve male infertility (Tempest et al, 2005).

There were more than 20 studies available on the treatment of male immune infertility with Chinese herbal medicine, which the Chinese seemed very used to treating. One study specifically looked at immune male infertility. The investigators used a combination approach, utilizing both acupuncture and herbs, which resulted in a significant decrease in AsAb. The investigators measured a “rate of 90% effectiveness” (Fu et al., 2005) resulting in pregnancy in the acupuncture-herb group, and a measurement of 64% effectiveness in the control group, which took prednisone (Fu et al., 2005).

One study (Tempest et al., 2005) reported a significant reduction in sperm disomy after treatment of herbs, but placebo-controlled clinical trials need to be done to affirm these findings. Sperm disomy is associated with smoking, advanced paternal age and compromised semen parameters: low concentration, motility and poor morphology. In fact, men with OAT (oligoasthenoteratozoospermia) have up to a 30-fold increase in disomy, and it is this population who is utilizing ICSI to overcome male-factor infertility. There are not many ways to screen semen for chromosome issues to date, and “clinical intervention has been limited to screening for defects with a view to genetic counseling of individuals with abnormally high levels about the risks of affected children and the likely success of the ICSI procedure itself” (Tempest et al., 2005).

Up until 2005 “there [had] been no studies reporting that these high levels [of disomy] in infertile men can, potentially, be reduced” (Tempest et al., 2005). This study by Tempest et al. (2005) clearly demonstrated that high chromosome abnormalities levels can be

reduced by herbal treatments. The authors propose that double-blind placebo-controlled clinical trials should ensue to test this hypothesis:

“Of course, good, well-designed studies should require no other justification; however, it is our opinion and experience that such a trial would be unlikely to be supported either financially or by ethical committees until a degree of evidence was found that suggested that the trial might be effective and this of patient benefit. Indeed [our colleagues have] emphasized the importance of open tube studies as forerunners for double-blind, placebo-controlled clinical trials of new pharmaceutical regimes; this was an example of such a study”

(Tempest et al., 2005).

Therefore, “given that the issue of the possible transmission of chromosomal abnormalities via ICSI raises significant concerns, any approach that was effective in reducing sperm disomy could ultimately be used prior to ICSI to improve success rates” (i.e. to increase the chances of injecting a chromosomally normal sperm) (Tempest et al., 2005).

The biggest issue to be tackled is the enormous complexity of the subject, as there are countless herbal remedies. In TCM single herbs can be used, as well as formulas, not to mention hundreds of TCM diagnoses that do not exactly correlate with Western diagnoses. In terms of testing, each herb may have a distinct set of bio-chemical

composition, and yet its effect on the human body may or may not change depending on what herbs it is cooked or ingested with. Tempest et al. (2008) made a good point, stating that “ it is conceivable that these complex decoctions may be capable of targeting and interacting with multiple signal transduction/endocrine/oxidant/metabolic pathways to effectively correct, counteract or circumvent the impaired or dysfunctional mechanisms of various male factor infertility phenotypes” (Tempest et al., 2008). The integration of Western and Eastern pharmacology holds a large potential, and the analysis and standardization of TCM herbal formulas is not a simple task (Tempest et al., 2008). Again, the 2008 study by Tempest et al. discusses the work ahead: “Isolating active compounds is similarly complex and any therapeutic efficacy of TCM may be related to the pharmacokinetic or pharmodynamic synergism of the ingredients with potentially many different effects contributing to the overall response. Moreover any potential effects may be the result of the metabolism of these compounds, the product of which may be responsible for the resultant activities” (Tempest et al, 2008). The chemicals from these herbs need to be identified and standardized, and geographical region, climate and season may all have an effect on these chemicals. Contamination must also be considered, such as pesticides, heavy metals, weeds, fungus or bacteria contamination and pollution from processing, especially as the majority of herbal products are unlicensed in most countries (Tempest et al., 2008). This has been, and will continue to be, a difficult subject to fully understand, and certainly will take time to test.

## **Other Observations**

There seemed to be more published articles and studies on herbs and male infertility than on acupuncture and male infertility, especially in the Chinese medical journals, with exception to the European journals.

In terms of study design, again, the individual studies shed light on the subject in a more direct way. The reviews simply summarize past studies and give an overall idea of what might be going on in current research. For example, Hung Yu Ng et al. (2008) did a systematic review of many studies which summarizes that there are not enough randomized studies (Hung Yu Ng et al., 2008). Another review states that pregnancy rates significantly increased in IVF when acupuncture was used on the day of embryo transfer (Deadman, 2008).

Also, the Chinese studies seem to follow couples after treatment more so than the US, European, Australian and Israeli studies in order to track conception rate and live births. Again, it may be worth tracking this factor in future studies as it is the ultimate goal, along with the health of mother, father and child.

## **DFI**

In terms of answering the hypothesis that TCM improves DFI, this literature synthesis can neither confirm nor contradict the hypothesis, as there were no published studies on

DFI and TCM therapies. What has been answered is that Chinese herbal medicine, whether used as a single herb or formula by the subject, or as an in vitro medium in which to directly affect the semen on contact, shows an overall improvement within many aspects of male infertility. Acupuncture therapy also has an overall positive effect on many sperm parameters, fertility markers and even pregnancy rates in some cases when measured. Thus, one may extrapolate that if the many aspects and measurements of male infertility are showing improvement with TCM (such as semen quality, leveling of hormones, regulation of the immune system and improvement in pregnancy or live birth rate), the DFI might be positively affected as well, although this has not been directly discovered as of 2011.

Many studies show that an improvement of DFI, i.e., a decrease in the DNA fragmentation of sperm, is often accompanied by an improvement in the quality of sperm. However an improvement of semen quality is not always coupled with an improvement in DFI, indicating that the standards for DFI may be higher, different or more specific than the traditional SA. As there is a growing number of studies showing that an improvement in DFI leads to an improvement in semen quality, embryo quality, and chances of pregnancy and live birth, one might project that DFI quality might be further studied, especially as it seems that DFI and SA may have different, but important, information to offer (Agarwaal & Said, 2003).

Up until 2008, “no major advances have been made in the medical management of poor sperm quality” except for the use of ICSI, which is not considered a cure (Sikka & Wang,

2008). When ICSI was developed male-factor infertility had a break-through in management of male infertility. However, this procedure does not treat the cause of infertility. Moreover, it may allow for inadvertently passing on adverse genetic consequences (Sikka & Wang, 2008). Using DFI measurements and sperm nuclear integrity assessments may be useful in the evaluation of unexplained male factor infertility especially as environmental chemicals may disrupt structural stability of sperm nuclei.

Sikka & Wang (2008) stated:

“[Evidence] suggests that damage to human sperm DNA might adversely affect reproductive outcomes and that the spermatozoa of infertile men possess substantially more sperm DNA damage than do spermatozoa of fertile men. This is particularly relevant in an era where advanced forms of assisted reproductive technologies are commonly used (technologies that often bypass the barriers to natural selection), because there is some uncertainty regarding the safety of using DNA-damaged spermatozoa. Evaluation of damaged sperm DNA seems to complement the investigation of factors affecting male fertility and may prove an efficient diagnostic tool in the prediction of pregnancy outcome” (Sikka & Wang, 2008).

The Sperm Chromatin Structure Assay (SCSA) is “likely to be the male fertility test of the 21<sup>st</sup> century,” (Deadman, 2008) just as semen analysis was for the 20<sup>th</sup> century. SCSA

is expensive and difficult to obtain, and measures something entirely different from the semen analysis, although the same factors that affect and create a poor SA may also affect DNA fragmentation, such as varicocele, age, smoking, heat, infection, toxins, etc. Although a measurement of more than 30% of DNA fragmentation is associated with the impairment of sperm's ability to fertilize the egg, reduction of the chance of a successful IVF/ICSI (Check et al., 2005), increase in abortion rates (Lin et al., 2007), and impairment of chances of a natural conception (e.g. Spano et al., 2000), the patient may have a normal semen analysis, and conversely, some men with poor semen analyses may have high DNA fragmentation indexes (Deadman, 2008).

Whether DFI actually does become the male fertility test of the 21<sup>st</sup> century or not, time will tell. Although the scientific community is divided, many fertility specialists participating in research are finding that examination of DFI shows worthwhile data concerning male infertility, as the DFI test seems to offer information which allows for better selection of sperm for use in IVF or ICSI, resulting in an increase in pregnancies and live births (Simon et al., 2010).

The next step would be to start conducting small pilot studies treating men with acupuncture and herbs and measuring the impact on DFI. Also, conducting larger scale studies using TCM therapies while using several measurements at once (hormone levels, immune markers, antioxidant effects, SA, pregnancy rate and live birth rate) would offer the most insight into TCM, DFI and male infertility. Also, tracking couples through to childbirth, the ultimate goal, would be a useful outcome measurement to include.

## **Implications for Theory**

Western medicine tends to treat female infertility more than looking to the male to treat male subfertility. Thus there are limited Western treatment options and the etiology is mostly unknown. In addition there is justification, as helping maximize female fertility will also help the male factor because an improvement in cervical mucus supports the sperm (Deadman, 2008). Cervical mucus not only protects the sperm and provides a place of survival for longer periods of time, but it also forms channels to advance quickly, and filters out sperm that have low motility and more morphological abnormalities, which only helps the highest quality sperm get to the egg sooner (Deadman, 2008). Uterine contractions, progesterone and glycoprotein ZP3 all assist in sperm activation and penetration (Deadman, 2008). Also, oocytes are able to repair sperm DNA damage, but this depends on the level of damage and the oocyte's capacity to repair and heal this damage (Nicopoullous, 2008). This suggests that no matter what abnormality the sperm has, the health of the female may be of more importance, as a stronger oocyte can repair sperm even better than a damaged or aged oocyte (Simon et al., 2010). Also, if the female partner is treated, regardless of which type of ART is used, often times ovarian stimulation is promoted, again, only making it easier for the sperm to perform. This literature synthesis will hopefully promote a shift in perspective on the importance of treating both male and female partners, and how easy, affordable and effective it can be to manage treatment of male infertility with the adjunctive use of TCM therapies (Deadman, 2008).

### *Chinese Herbal Medicine*

The TCM diagnosis of Kidney Yang deficiency is often made when a man is diagnosed with infertility. Kidney Yang deficiency may be associated with testosterone deficiency, or the many different mechanisms within spermiogenesis, affecting azoospermia or asthenospermia. The traditional manifestations of Kidney Yang deficiency may be expressed by having soreness and weakness of the lumbar region, urinary incontinence, enuresis, nocturnal urination, reproductive issues, along with a pale, white-coated tongue, and a weak or thready pulse. TCM tends to interpret non-specific male infertility as Kidney Jing or Essence deficiency, as it is usually attributable to an “energetic failure of the Kidney” (Clicci et al., 2009). In Western terminology, Kidney deficiency may generally be associated with hormonal imbalances, while Damp-Heat syndromes are associated with inflammation of the genital tract, and Kidney-Yang deficiency with spermatogenic failure (Siterman et al., 2009). A review done in 2003 (Xu et al., 2003) shows many correlations between the Chinese diagnosis of Kidney deficiency and the Western diagnosis of poor sperm quality, so this may be a topic worth exploring in terms of TCM theory and practice (Xu et al., 2003).

An interesting connection between Western and Eastern TCM diagnoses and formula treatments was the use of Shao Fu Zhu Yu Tang in the treatment of chronic prostatitis and poor SA. According to TCM, this formula is traditionally given for Blood Stagnation in the abdomen. Both Western diagnoses (prostatitis and poor semen quality) may be caused by Blood Stagnation. They may also be caused by a variety of Western

mechanisms of pathophysiology. In one study (Yang et al., 2003) the formula moved the stagnant Blood, and by doing so, not only treated the prostatitis, but sperm count, motility and morphology improved, improving overall semen parameters. Regardless of the actual cause of poor SA and prostatitis in this case's subjects, perhaps the act of moving Blood was enough to cause a positive shift in the majority of the patients treated. This formula also decreased free radicals, indicating a possible mitigation of oxidative stress, and may be useful in cases where oxidation levels are too high and antioxidants are needed to balance out the system. Perhaps these subjects specifically had higher amounts of reactive oxygen species (ROS) either caused by or attributing to the chronic state of inflammation in the reproductive system. It might have been the antioxidative effect of this formula that helped clear the symptoms, improve sperm quality and move Blood. Or it might have been a combination of effects that improved the outcome in this study (Yang et al., 2003).

Sometimes different TCM diagnostic formulas are used for the same western diagnosis. Conversely, sometimes only one TCM formula will be used for more than one Western diagnosis. For example, the Shao Fu Zhu Yu Tang formula mentioned above is also used to treat other symptoms, such as abdominal masses like fibroids or PCOS. In the previous study (Yang et al., 2003) it was used to treat prostatitis and poor semen quality.

Another example of one formula being used to treat more than one Western symptom or diagnosis would be the herbal formula Bu Zhong Yi Qi Tang. This formula was used in a study by Amano et al. (1996) as an in vitro experiment to see how it might affect semen

quality. The formula increased sperm motility as well as seminal plasma levels which correlate with concentration (Amano et al., 1996). The same formula was used a decade later by Akashi et al. (2006) in 20 men to test its in vivo effects on basic semen parameters such as motility, morphology and count. The formula showed an increase in sperm motility, although count, volume and morphology did not change (Akashi et al., 2006; Yang et al., 2003).

As an example of many TCM formulas used to treat one single Western diagnosis, the many reviews considered in this study showed that the Western diagnosis of “Male Infertility” may be treated with several different Chinese medical formulas, such as Shao Fu Zhu Yu Tang, Bu Zhong Yi Qi Tang, Liu Wei Di Huang Tang or Ju Jing powder. The key for differentiation is not only the underlying Western cause for sperm quality (stress, APA, hormone imbalance, history of prostatitis or illness, immune system imbalances, or a history of physical trauma or surgery) but also the TCM diagnosis (such as Kidney Yang Deficiency, Damp-Heat in the Lower Jiao, or local Qi and Blood Stagnation, etc.) (Xu et al, 2003; Deadman 2008; Lyttleton 2005).

The above implies that there is tedious work ahead. Each single herb used, whether in a formula or alone, may have a potential hormonal or immune-related effect. For example, Yin Yang Huo was shown in one study to “improve Kidney Yang”, its Chinese medicine function (Xu et al., 2003). In another study this same herb was shown to improve FSH, LH and testosterone levels (Crimmel et al., 2001).

The concept of Du Yao (herbs paired together in groups of two) complicates the situation further. Single herbs may have a different effect once paired. Their combination may increase or diminish or synergistically change their impact, than if used alone (Sionneau, 2002). There are hundreds of formulas and various modifications using these Du Yao pairings, chosen by an individual practitioner which introduces yet another variable. There are a vast amount of possibilities in herbal medicine, and this certainly contributes to the complexity of future research of traditional Chinese herbal medicine.

### *Acupuncture Therapy*

In terms of theory, the acupuncture therapy implications for the results of this literature synthesis may be more clear-cut. Acupuncture is safe and seems to be efficacious on multiple levels, especially if used in a frequent course of treatment. One investigator concludes, “the exact mechanism by which acupuncture treatment decreases necrospermia and asthenospermia remains unclear. However, since both sperm motility and axonema impairment are known to be linked with lipid peroxidation in human spermatozoa, it is possible that the treatment is related to the reduction of peroxidation processes” Sherman et al. continues, “In conclusion, subfertile patients exhibiting low sperm activity seem to be good candidates for acupuncture treatment” (Sherman et al., 1997).

After reviewing the studies, it would seem that no matter what the TCM diagnosis and treatment protocol was, there was at least some increase in semen quality (Zhiyuan, 1996;

Sherman et al., 2007). What may be of more concern is the frequency of treatment. For example, the 2008 case study (Cliaci, 2008) showed that the subject observed received treatment (no specific points were stated, but the Kidney meridian was targeted) every 2-3 days, up to 10 treatments total. The improvement in sperm count and density was a 90-fold increase, with count moving from 0.54 million to 46.5 million and density increasing from 0.2 million/mL to 18.6 million sperm/mL (Cliaci, 2008).

The age a man is diagnosed as infertile may be one of the most difficult conditions for TCM to treat. One study in 2003 (Gurfinkle et al., 2003) tested acupuncture with moxa on 19 subjects who had not fathered children for eleven years, and who had been diagnosed with semen abnormalities in concentration, morphology and motility.

Although there was an increase in normal form sperm, other parameters such as volume, concentration, progressive motility and number of round cells (could be WBC's, possibly indicating infection, immature sperm or other cellular components) did not show significant changes (Gurfinkel et al., 2003).

Another interesting finding is that some of the acupuncture treatments were short courses, consisting of treatments twice a week for five weeks. Still, they created a change significant enough to make a difference in most cases, with improved sperm count, motility, viability and intactness of axonema (Siterman et al, 2007), which means within 35 days of treatment, there was a moderate, but quick effect. If spermatogenesis takes approximately 70-90 days, this implies that a longer course of treatment may have an even more positive effect on sperm quality and potentially on pregnancy and live birth

rate. It also implies that the best time for a man to get acupuncture treatment may be the week preceding his female partner's ovulation, as it seems to have an effect quicker than expected. Moreover, if the patient is willing to take herbs for three months and then have acupuncture treatment several weeks preceding ovulation, this might have a synergistic effect and create an even more positive outcome than improved sperm quality, such as pregnancy and live birth rate, whether trying to conceive naturally or with ART.

And finally, in one study, men with varicocele showed little to no improvement in SA after the acupuncture treatment, even though it is believed that when there is ROS-mediated damage to the sperm count, acupuncture would interrupt the peroxidation process and thereby improve sperm quality (Siterman et al, 2009). More studies need to be done. Another interesting result in this study concerns subjects who presented with both inflammation and high FSH, and only half of these men showed improvement in sperm quality. There was an increase in integrity and concentration of sperm with the acupuncture treatment. This suggests that if there is a mixed etiology, perhaps one needs to find the dominant etiology and treat that first (Siterman et al., 2009).

## **Implications for Practice**

### *Acupuncture Therapy*

For Acupuncturists, the results indicate that frequent treatments, i.e. 1-2 times weekly for a minimum of five weeks may increase sperm quality and other imbalances related to

infertility or subfertility. This idea agrees with what Sherman et al. (1997) and Siterman et al. (2007) found. Therefore, the way acupuncturists practice may shift according to the findings of this literature synthesis. For example, perhaps a short, but frequently timed course of treatment may be sufficient for individuals with inflammation or prostatitis, whereas longer courses of treatment may be more advisable if the diagnosis or symptom picture is more complex, such as an additional diagnosis of APA, an exposure to radiation, or a history of varicocele.

In the case of idiopathic male infertility one case (Zhang et al., 2002) reported no improvement of sperm concentration. In this study, subjects had a history of one failed ICSI. After acupuncture therapy, form and motility improved, and fertilization improved from 40.2% to 66.2%, raising the question why researchers and medical practices are not focusing on a more integrative approach, especially as form, and possibly motility, are just as important when using ART as the more motile sperm may be stronger or healthier, and form or morphology are important in forming a viable embryo.

### *Chinese Herbal Medicine*

In TCM there are certain herbs that are known to help sperm quality overall, such as Huang Qi or Tu Si Zi. These herbs increase “Qi” and “Tonify Kidney Qi”, respectively (Amano et al., 1996; Liu et al., 2004; Xu et al., 2003). Other herbs and formulas treat according to a constitutional TCM diagnosis. An example is Shan Zhu Yu, which is used to “stabilize the Essence or Jing, and tonify the Kidneys and the Liver”. In Yang’s 2003

study, this herb is used not to overtly treat a man's constitution, but to treat a large number of men who have prostatitis and poor SA, and it did so effectively. This affects the traditional Chinese herbalist in practice because it means that not only do herbal formulas affectively treat specific, correlated diagnoses (Kidney Essence or Yang deficiency and poor sperm quality) but that each single herb may have a chemical component that treats unexpected Eastern Medicine conditions or more than one allopathic symptom or condition. The implication here is that TCM herbalists must remain open-minded to the results of Western investigations into the chemically based, proven effects of herbs. Another implication might be that single herb use may increase in practice with such knowledge (Yang et al., 2003).

In terms of integrating both Eastern and Western practices, there seems to be potential for a harmonious and working relationship between both Western and Eastern fertility specialists. For example, the subjects in most of the studies included in this literature review showed an improvement in one or more aspects of their male infertility diagnosis in response to TCM herbal treatment, whether trying to conceive naturally or with the aid of ART. There is medical evidence for TCM treatment modalities supporting Western fertility treatments in increasing fertility and improving ART outcomes, as demonstrated in this literature review and synthesis (Zhang et al., 20020; Deadman, 2008; Hung Yu Ng et al., 2008; Huang & Chen, 2008).

Western fertility specialists may want to consider referring their male patients to a TCM practitioner. Alternatively, there may be TCM practitioners on site so that the process

may be streamlined and more convenient and less stressful for the patient. Also, the reduction of the patient's stress often accomplished with acupuncture may be beneficial for MD's, as tests and appointments may also be beneficially affected. Herbal medicine also seems to have potential in a more integrative setting. Perhaps Andrologists will begin to test and incorporate the in vitro Huang Qi treatment that seemed to repeatedly show benefit (Amano et al., 1996; Liu et al., 2004).

Other MD's may want to consider referring their patients to a Chinese herbal medicine fertility specialist for an evaluation and different perspective to address any other underlying emotional or physical, systematic or local conditions. Some of the basic constitutional formulas may correct subtle imbalances that improve semen quality and chances of conception (e.g., heat-clearing or immune balancing formulas), while some specific herbs may help with sperm count or motility specifically. For example, the combination study (Fu et al., 2005) used acupuncture and herbs versus oral prednisone to treat immune infertility. The "positive rate of blood serum and/or AsAb in both groups decreased in varying degrees, but the negative-turning rate of AsAb in the acupuncture-herb group was more obvious, the comparison showing also a significant difference ( $P < 0.05$ )" (Fu et al., 2005) suggesting that having a holistic approach which treats the individual's overall condition with TCM may be of very much help (Fu et al., 2005).

### **Limitations of the Current Study**

In terms of acupuncture, it was interesting to note that one European study on acupuncture and male infertility was from the early 1980's. It was not included in this analysis because it was not within the timeframe as given by the inclusion criteria. In future literature reviews the time frame should perhaps be extended to include these earlier studies.

Animal studies were not included. It may be beneficial to also look at animal studies as it may be an easier way to test the effects of herbs on spermatogenesis, hormone levels and fertilization.

Studies revolving around the investigation of nutritional supplements or antioxidants and sperm quality were not included, as the main research question addressed in this study was related to TCM in its most holistic form. Although, there may be significant antioxidant activity in some of the herbs and formulas used in the analyzed studies, this was not the focus of the study. This should be investigated in a future study.

### **Inclusion**

Of 35 articles in Chinese language found, only six studies of similar subject were included in this analysis in order to include more studies on herbal medicine as there are few available in the English language. Also, including some Chinese language articles

may help give some idea as to what has been done, even if the studies are not exactly at the same level as English language studies. As there is so little information, the decision to include a small sample of Chinese language studies might be helpful to gain a better understanding of the concept. Part of the reason for excluding most Chinese studies was the language barrier, lack of translational work, lack of funding to purchase large amounts of translated work, and the inability to grade a study's quality as China has different ways of conducting studies. It also seems the studies from China show a trend in that not all herbs were disclosed in a given formula used. Some studies listed a few herbs and then continued with "etc." indicating that there were more herbs that were not listed (Zhiyuan, 1996).

Although including reviews is discouraged in current Literature Syntheses for the most part, review articles were included because these reviews gave important information and therefore potential insight that would have been overlooked otherwise. The other reason for including reviews is the general lack of studies in this area. At this juncture, specializing scientists, Urologists, Andrologists, OBGYN's, RE's, Immunologists, Integrative fertility specialists and, most of all, concerned patients need all the help they can get. Thus, including all pertinent information in this analysis may eventually lead to more efficient, less costly and overall better care, and is of necessity. For example, one case study that was discussed in a review reported a very high rate of pregnancies (55.6%) after only three courses of treatment, which in this study was approximately 30 treatments total. This study suggests that acupuncture may be effective in improving chances of pregnancy in male infertility with a minimum of 30 treatments required

(Zhiyuan, 1996). Another study discussed in a review reported a 78% pregnancy rate in available couples for questioning. All subjects consumed a Chinese herbal remedy twice a day for 60 days (Crimmel et al., 2001). Both of these studies were discussed in reviews and show that TCM was efficacious in safely treating male infertility.

### **Recommendations for Future Research**

To the best of my knowledge there were no studies published that actually used DFI as an outcome when testing the effects of acupuncture or herbs on semen quality and male factor infertility. This leaves a hole in current research, suggesting the possible need for future investigation.

In terms of acupuncture, there needs to be more rigorous, high-quality studies that include a higher number of subjects, as well as an effort to follow-up and track couples to procure live birth rates. Hopefully, in the future, studies will include a larger number of men in order to get a better sense of the effectiveness of TCM treatment modalities in the overall infertile and sub-fertile population. Also, the concept of TCM diagnosis needs to be further explored, whether an individual is going to be treated with acupuncture constitutionally, treating the root, or based on a branch diagnosis and current symptoms.

For herbal medicine, research may be more complicated as there are hundreds of herbs, hundreds of formula combinations, numerous locations of plant growth, harvesting methods, regulations, processing and consumption methods. Studies on plant assays,

single herbs, formulas, and in vivo or in vitro need to be done. Perhaps in the future it would be useful to test both in vitro and in vivo effects of herbs on one specific parameter of male infertility, such as sperm motility and morphology, while also tracking long-term outcome measures such as live birth rates.

One study (Yuan & Lin, 2000), done by the Department of Cell Biology and Molecular Genetics at University of Maryland, looked at the use of Chinese herbal medicine to treat asthma, dermatitis and IBS. Although the topic is not the same as male infertility, the concept of researching these herbal formulations in a rigorous approach reported clinically validating their uses by including chemical standardization, biological assays, animal models, and clinical trials. The authors were able to scientifically prove which formulas worked. The investigators' advice was clear: "Such Western methodologies need to take into consideration the complex mixture of chemicals and how they are to be used in humans" (Yuan & Lin, 2000).

The classical Chinese pharmacopoeia holds a vast amount of formulations, which treat a wide variety of illnesses and symptoms. Although the approach of using TCM herbal formulas is often ignored, an interdisciplinary approach to TCM may provide a platform for the discovery of novel therapeutics composed of multiple chemical compounds (Yuan & Lin, 2000).

It would be interesting, and hopefully not very expensive, to track pregnancies or live birth rate results in response to TCM treatment for subfertile men and perhaps follow the

couples long enough to discover findings concerning the child's health, especially as there has been some inquiry concerning the health of the embryo conceived with ART. Moreover, studies show a relationship between DFI and embryo quality. Nicopoulos et al., (2008) reported that "no pregnancy was reported using ART when DFI of the raw semen sample was >27%, and DNA fragmentation was suggested to affect the outcome of IUI cycles, IVF and ICSI fertilization, embryo development, embryo quality, implantation and the pregnancy outcome of ART" (Nicopoulos et al., 2008). This study confirms a relationship between male subfertility and sperm DFI, and suggests that the next line of thought would be finding new techniques for diagnosis and treatment of male subfertility and sperm DNA fragmentation. Perhaps a study on possible integrative treatments utilizing all of these measurements (SA, DFI, hormone levels, antioxidants and live birth) would be helpful and result in correlating this information into useful clinical knowledge. (Marchesi & Feng, 2007).

More studies need to be done in hopes to not only treat male infertility successfully, but to promote conscious conception and the creation of healthy children who do not develop serious health concerns in their future. This is especially important as the field of epigenetics is increasingly showing the importance of pre-conception care, and the effects of dietary intake, stress, chemicals and lifestyle on both embryo quality and health of the child (Segars et al, 2009). Another benefit of utilizing an integrative treatment approach would be to help both parents (or, gamete donors) to reduce their hormone therapy intake during ART, and experience fewer and less expensive, inconvenient, painful and invasive

interventions, such as ICSI, or at the very least improve chances of conception and live birth with ICSI, along with increasing chances of birth of a healthy child.

## **Conclusion**

To the best of my knowledge, currently, there have not been any studies published on potential effects of TCM treatment modalities on DFI. Investigations are strongly recommended. TCM was shown to improve semen parameters other than DFI, including morphology, motility, concentration, volume and count. Also, hormone levels, quality and quantity of immune markers, antioxidant levels, which are all related to sperm quality, tended to improve with TCM treatments. The next step is to complete a research study on male infertility subjects testing SA, hormones and DFI before and after a course of TCM treatment modalities, including acupuncture and herbal medicine. Facilitating fertilization, pregnancy, live birth rates and healthy children via natural conception or ART is the ultimate goal when it comes to improving semen parameters, and TCM may improve this facilitation. Larger, more controlled studies need to be done.

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## Appendices

Table 1. TCM Treatment Modalities and Conception Methods

		TCM Treatment Modalities and Conception Methods						
		Acupuncture		Combination	Herbs		Conception Method	
Articles		Acupuncture	Acupuncture & Moxibustion	Acupuncture & Herbs	Internal Consumption (In Vivo)	Sperm Medium (In Vitro)	ART	Natural
acupuncture	1	Clinical Observation of 54 Cases of Male Infertility Treated by Acupuncture and Moxibustion. Zhiyuan, 1996	X	X				
	2	Effect of Acupuncture on Sperm Parameters of Males Suffering from Subfertility Related to Low Sperm Quality. Sherman, et al. 1997	X					
	3	Does acupuncture treatment affect sperm density in males with very low sperm count? A pilot study. Siterman, et al. 2000	X					
	4	Influence of acupuncture on idiopathic male infertility in assisted reproductive technology. Zhang, et al. 2002	X				X	
	5	Effects of acupuncture and moxa treatment in patients with semen abnormalities. Gurfinkel, et al. 2003	X	X				
	6	Quantitative evaluation of spermatozoa ultrastructure after acupuncture treatment for idiopathic male infertility. Pei, et al. 2005	X					
	7	The Treatment of Male Subfertility with Acupuncture. Deadman, 2008	X	X			X	X
	8	The role of acupuncture in the management of subfertility. Hung Yu Ng, et al. 2008	X				X	
	9	Acupuncture for the Treatment of Cryptozoospermia. Cliaci, 2008	X					
	10	Acupuncture Treatment of Idiopathic Male Infertility. Claici, et al. 2009	X					
	11	Success of acupuncture treatment in patients with initially low sperm output is associated with a decrease in scrotal skin temperature. Siterman, et al. 2009	X					
	12	A prospective randomized placebo-controlled study of the effect of acupuncture in infertile patients with severe oligoastheno-zoospermia. Dieterle, et al. 2009	X					
combo	13	Withered Yang: A Review of traditional Chinese Medical Treatment of male Infertility and Erectile Dysfunction. Crimmel, et al. 2001	X	X	X	X	X	
	14	Effects of combined therapy of acupuncture with herbal drugs on male immune infertility – a clinical report of 50 cases. Fu, et al. 2005	X		X	X		

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## TCM Treatment Modalities and Conception Methods

Articles		Acupuncture		Combination	Herbs		Conception Method	
		Acupuncture	Acupuncture & Moxabustion	Acupuncture & Herbs	Internal Consumption (In Vivo)	Sperm Medium (In Vitro)	ART	Natural
herbs	15	TCM treatment of male infertility due to seminal abnormality – a clinical observation of 82 cases. Zhai Yachun, et al. 1990				X		
	16	Effects of Chinese herbal medicine on sperm motility and fluorescence spectra parameters. Amano, et al. 1996					X	
	17	A substance isolated from <i>Corus offinalis</i> enhances the motility of human sperm. Jeng, et al. 1997					X	
	18	Effects of Shao Fu Zhu Yu Tang on motility of human sperm. Yang, et al. 2003				X		
	19	Application of Traditional Chinese medicine in the treatment of Infertility. Xu, et al. 2003				X	X	X
	20	Effects of several Chinese herbal Aqueous extracts on human sperm motility in vitro. Liu, et al. 2004					X	
	21	Significant reduction of sperm disomy in six men: effect of traditional Chinese medicine? Tempest, et al. 2005				X		
	22	Effect of herbal medicine bu-zhong-yi-ki-tang on semen parameters and seminal plasma cytokine levels in idiopathic male infertility. Akashi, et al. 2006				X		
	23	TCM treatment of male immune infertility – a report of 100 cases. Sun, et al. 2006				X		X
	24	Plants Used in Chinese Medicine for the Treatment of Male Infertility Possess Antioxidant and Anti-Oestrogenic Activity. Tempest, et al. 2008*					*	
	25	Traditional Chinese medicine and infertility. Huang & Chen, 2008.				X		X
	26	Withania somnifera improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. Ahmad, et al. 2010				X		
	27	[Effect of modified tianxiong powder in treating oligospermia and asthenospermia] Geng, et al. 2010				X		

\* Plants were measured for endocrine and antioxidant activity. Not consumed by subjects or used in vitro on semen.

Review of several studies.

Table 2. Measurements Used to Assess Effects of TCM on Semen Quality and Male Factor Infertility

Articles		Biomarkers / Tests / Success						
		Semen (Parameters or SA)	DNA Fragmentation Index (DFI)	Hormone/Immune Markers	Antioxidants	Fertilization	Pregnancy	Live Birth
1	Clinical Observation of 54 Cases of Male Infertility Treated by Acupuncture and Moxibustion. Zhiyuan Q. 1996	X						X
2	Effect of Acupuncture on Sperm Parameters of Males Suffering from Subfertility Related to Low Sperm Quality. Sherman S, et al. 1997	X [+ "Fertility Index"]**			X [zinc & citric acid]		X [30%]	
3	Does acupuncture treatment affect sperm density in males with very low sperm count? A pilot study. Siterman S, et al. 2000	X						
4	Withered Yang: A Review of traditional Chinese Medical Treatment of male Infertility and Erectile Dysfunction. Crimmel A, et al. 2001 (Several Studies)	X		X [CHM]	X[CHM]			X
5	Influence of acupuncture on idiopathic male infertility in assisted reproductive technology. Zhang M, et al. 2002	X				X	X	
6	Effects of acupuncture and moxa treatment in patients with semen abnormalities. Gurfinkel E, et al. 2003	X						
7	Quantitative evaluation of spermatozoa ultrastructure after acupuncture treatment for idiopathic male infertility. Pei J, et al. 2005	X						
8	The Treatment of Male Subfertility with Acupuncture. Deadman P 2008 (Several Studies)	X	Mentioned but not Measured					X
9	The role of acupuncture in the management of subfertility. Hung Yu Ng, et al. 2008 (Several studies)	X		X				X
10	Acupuncture for the Treatment of Cryptozoospermia. Cliaci D. 2008	X						
11	Acupuncture Treatment of Idiopathic Male Infertility. Claici D, et al. 2009	X		X [PRL,T]				
12	Success of acupuncture treatment in patients with initially low sperm output is associated with a decrease in scrotal skin temperature. Siterman S, et al. 2009	X		X [FSH]				

Table 2. Measurements Used to Assess Effects of TCM on Semen Quality and Male Factor Infertility

Articles		Biomarkers / Tests / Success						
		Semen (Parameters or SA)	DNA Fragmentation Index (DFI)	Hormone/Immune Markers	Antioxidants	Fertilization	Pregnancy	Live Birth
13	A prospective randomized placebo-controlled study of the effect of acupuncture in infertile patients with severe oligoastheno-zoospermia. Dieterle S, et al. 2009	X						
14	TCM treatment of male infertility due to seminal abnormality – a clinical observation of 82 cases. Zhai Yachun, et al. 1990	X						
15	Effects of Chinese herbal medicine on sperm motility and fluorescence spectra parameters. Amano T, et al. 1996	X [+plasma: sFas***]						
16	A substance isolated from Cornus officinalis enhances the motility of human sperm. Jeng H, et al. 1997	X						
17	Effects of Shao Fu Zhu Yu Tang on motility of human sperm. Yang CC, et al. 2003	X [+ acrosin levels]		X [FSH, LH, T, corticosterone]	X [serum free radicals]			X
18	Application of Traditional Chinese medicine in the treatment of infertility. Xu X, et al. 2003	X [+ sperm capillary penetration; hypo-osmotic swelling]						
19	Effects of several Chinese herbal Aqueous extracts on human sperm motility in vitro. Liu J, et al. 2004	X [motility]						
20	Significant reduction of sperm disomy in six men: effect of traditional Chinese medicine? Tempest H, et al. 2005	X [disomy]						
21	Effects of combined therapy of acupuncture with herbal drugs on male immune infertility – a clinical report of 50 cases. Fu B, et al. 2005			X [AsAb]				
22	Effect of herbal medicine bu-zhong-yi-ki-tang on semen parameters and seminal plasma cytokine levels in idiopathic male infertility. Akashi T, et al. 2006	X						
23	TCM treatment of male immune infertility – a report of 100 cases. Sun Z, et al. 2006	X		X [AsAb]				X

Table 2. Measurements Used to Assess Effects of TCM on Semen Quality and Male Factor Infertility

**Biomarkers / Tests / Success**

Articles		Biomarkers / Tests / Success						
		Semen (Parameters or SA)	DNA Fragmentation Index (DFI)	Hormone/Immune Markers	Antioxidants	Fertilization	Pregnancy	Live Birth
24	Plants Used in Chinese Medicine for the Treatment of Male Infertility Possess Antioxidant and Anti-Oestrogenic Activity. Tempest HG, et al. 2008*			X [endocrine assay]*	X [antioxidant assay]*			
25	Traditional Chinese medicine and infertility. Huang & Chen, 2008.			X	X		X [implantation]	
26	Withania somnifera improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. Ahmad MK, et al. 2010	X		X	X [oxidative biomarker]			
27	[Effect of modified tianxiong powder in treating oligospermia and asthenospermia] Geng Q, et al. 2010	X						

\*Endocrine and Antioxidant markers from plant assays. Not measured from blood serum levels.

\*\* FI (Fertility Index) was significantly associated with all acupuncture-affected sperm variables, i.e., percentage of viability, total motile spermatozoa per ejaculate, TFSF (total functional sperm fraction: count, motility & morphology ratio), and axonema integrity. This team developed FI based on a proportional combination of selected parameters and is "highly accurate in classifying of fertile and subfertile males (97% sensitivity and 90% specificity)" with an 80% correct mean prediction ability.

\*\*\*Correlated with concentration.

CHM: Chinese Herbal Medicine

PRL: Prolactin

T: Testosterone

FSH: Follicle Stimulating Hormone

LH: Leutinizing Hormone

AsAb: Antisperm Antibodies

Disomy: an extra chromosome in the sperm head

■ : Review of several studies.

■ : Combination of Acupuncture and Chinese herbal medicine in a single study.

Table 3. Methods and Results in Studies Evaluating Use of Acupuncture to Treat Male Infertility

Articles		Methods and Results					Semen Quality		Fertilization, Pregnancy or Live Birth Rate
		Study Design	n	Duration of Treatment	Number of Points	Points Used (Bilateral)	Before	After	
1	Zhiyuan Q 1996	Case Reviews	54	10 Treatments @ 30 min. 1-3 courses @ 10 treatments per course. Ave = 20.5 treatments.	6	Ren 3, 4, ST 36, SP 6 with Moxa	Men with diagnosed abnormal semen parameters, ejaculatory or erectile dysfunction.	24.07% showed improvement (increase in count & motility) 20.27% cases were ineffective (no pregnancy or improvement in SQ)	30/54 (55.6%) got pregnant after 3 courses (30 tx's)
2	Sherman et al 1997/Siterman et al 1997	Prospective nonrandomized case-control study	16	2x/wk for 5 wks	Each subject received their own combination.	Chosen from: LU7,LI4,LI11,ST30,ST36,SP6,SP9,SP10,HT7,B20,23,33,K6,7,P6,LV5,8,R1,2,4,6,DU4	Subjects had "failed natural conception" for about 5 years. 6% oligozoospermia, 6% teratozoospermia, 44% asthenozoospermia, 25% asthenoteratozoospermia, 6% oligoteratozoospermia, 13% OTA syndrome. All participants had some bacterial contamination and presence of WBCs. About 50% had either hx of vericocele or prostatitis. Sperm viability 52%; Intact axonema 32%; Count 8.5 10 <sup>6</sup> /mL.	Increase in "Fertility Index" (p<.05). Significantly higher values of 3 basic sperm parameters: Percentage of viability, Total # of motile spermatozoa & TFSF (mean Total Functional Sperm Fraction: count, motility, morphology). Sperm viability 65%; Intact axonema 51%; Count 19.3 10 <sup>6</sup> /mL.	4 spontaneous pregnancies (30%) were achieved following the acupuncture treatment. (Not to be used as a criterion since female infertility was not ruled out).
3	Siterman et al 2000	Prospective controlled Pilot Study	40	2x/wk for 5 wks	A customized selection from the points used. No more than 12 points were punctured during a single session.	KD xu (assoc w hormonal imb) and DH (assoc w inflm of gen tract) were used: SP6, R4, LU7, K6, ST30. Add'l:K3, UB23, K11, UB52 (KD yang xu (assoc w spermatogenic failure). *	Low sperm count. Azoo- & Oligospermia.	13 of 20 had an increase in sperm density. 67% had increase in count.	Two couples underwent ICSI and both conceived.

Table 3. Methods and Results in Studies Evaluating Use of Acupuncture to Treat Male Infertility

Articles		Methods				Semen Quality		Fertilization, Pregnancy or Live Birth Rate	
		Study Design	n	Duration of Treatment	Number of Points	Points Used (Bilateral)	Before		After
4	Crimmel et al 2001	Uncontrolled Case Reports	6-248	Varied: 20 days to 18 weeks	Varied: 13 was highest amount. 4 was the lowest amount.	Not reported	Low motility, Low count, Low density, Low viability	Statistically significant improvement	Varies from 'Not Reported' to 74% of cases either had pregnancy or normilization of semen parameters
5	Zhang et al 2002	Nonrandomized case control studies	20	2x/wk for 8 weeks	unknown	unknown	Idiopathic male infertility with 1 failed ICSI.	Form and post-acup rapid sperm motility improved, as well as fertilization. Normal form sperm ratio increased. Concentration did not improve.	Fertilization improved from 40.2% to 66.2%
6	Gurfinkel et al 2003	Prospective, randomized, controlled, single-blind.	19	2x/wk for 10 weeks	E30,E36,BP6,F3,R3,IG4,BP4,CS6	Acupuncture with moxa	11 years w/o children, with semen abnormalities in concentration, morphology and motility	Increase in percentage of normal-form sperm. Volume, concentration, progressive motility and # of round cells did not show significant changes.	N/A
7	Pei et al 2005	Prospective controlled study	40	2x/wk for 5 wks	18	R4, UB23,32, LV3, K3, ST36, SP6, SP10, ST29, DU20	Idiopathic infertility for 2 years with 2 pathological spermograms (oligo-, astheno-, and/or teratozoospermia)	In in Morph & Motility	N/A
8	Deadman 2008	Review/Discussion	N/A	N/A	N/A	N/A	N/A	N/A	N/A
9	Hung Yu Ng et al 2008	Systematic Review	N/A	N/A	N/A	N/A	N/A	N/A	Authors state there are not enough randomized studies; however, pregnancy rate of IVF was higher when acupuncture was given day of ET.
10	Cliaci 2008	Case Study	1	10 tx's in 2-3 day intervals	unknown	Kidney Meridian Points	Vol 2.7 mL, Count 0.54 million, Density 0.2 million/mL	Count 46.5 million, Density 18.6 million sperm/mL, a 90-fold increase.	Not measured

Table 3. Methods and Results in Studies Evaluating Use of Acupuncture to Treat Male Infertility

Articles		Methods					Semen Quality		Fertilization, Pregnancy or Live Birth Rate
		Study Design	n	Duration of Treatment	Number of Points	Points Used (Bilateral)	Before	After	
11	Cliaci et al 2009	Prospective Non-randomised	45	10-15 tx's in 2-3 day intervals	20	LV3, SP6, 8, 9, R2, 4, DU4, UB 23, 52, 31, 32	Diagnosed w idiopathic male infertility. Excluding vericocele/prostatitis.	Marked increase in all parameters: count/ejaculate rose 3 to 10-fold. Functional sperm rose 2.5 to 3 times. Mobile sperm increased 3-4 times. Morphology rose 3% to 12%. Some had a dec in Prolactine and an increase in Testosterone.	12 pregnancies & births.
12	Siterman et al 2009	Prospective Non-randomised	39	8-10 treatments, 2x/wk.	A customized selection from the points used. No more than 12 points were punctured during a single session.	KD xu (assoc w hormonal imb) and DH (assoc w inflm of gen tract) were used: SP6, R4, LU7, K6, ST30. Add'l: K3, UB23, K11, UB52 (KD yang xu (assoc w spermatogenic failure). *	Low count & elevated scrotal temperatures, high gonadotropins/mixed etiology.	No improvement in Vericocele pts. Increase in integrity overall. Dec in scrotal temp. Inc in concentration. Mixed results when both inflammation and high FSH present.	Not measured.
13	Dieterle et al 2009	Prospective, randomized, single-blind, placebo-controlled.	52	2x/wk for 6 wks @ 45min	17	ST36, SP6, K3, LV3, UB 23, UB32, ST29, SP10, R4	Severe oligoasthenozoospermia and/or Infertility  (Concentration < 1 million)	Increase in %age of motile sperm, but no effect on concentration.  Dec in Vol. Inc in Conc w placebo.	Not measured.
14	Fu et al 2005	Randomized: Combined Acupuncture & Herbal Medicine study	100	not reported	16	UB15, 17, 18, 23, LV3, K3, HT7, SP 10	Subjects diagnosed with Infertility and Elevated AsAb	Significant decrease in AsAb. 90% effectiveness in acup-herb (LWDHW) group. 64% effectiveness in control group (oral prednisone).	N/A

\* Additional points used: SP9, LV5, LI11, ST28, GB41 (only for inflammation in genital tract). Secondary points: LI4, ST36, SP10, HT7, UB20, P6, R1, 2, 6, DU4, 20, GB20, LV3, K7, GB27 according to TCM principles and individual need. No more than 12 points were used in single session.

 Review of several studies.

 Combination study using both acupuncture and Chinese herbal medicine.

Table 4. Methods and Results in Studies Evaluating Use of Chinese Herbal Medicine to Treat Male Infertility

**Methods and Results**

Articles		Study Design	n	Duration of Treatment	Single Herb or Herbal Extract	Herbal Formula	Semen Quality		Pregnancy Rate	
							Before	After		
in vitro	1	Zhai Yachun et al 1990	Case Studies	82	N/A (several studies)	Fx consists of: Shu Di, Gou Qi Zi, He Shou Wu, Ze He Che, Yin Yang Huo, Sha Yuan Ji Li, Fu Ling, Yu Zhu, Yi Yi Ren and "with additions".	Ju Jing powder	unknown	Increase in motility, count & density - and "effective rate of 85.4%"	N/A
	2	Amano et al 1996	Non-randomised controlled study	14	1-6 hours incubation.	Chinese name of Fx: Bu Zhong Yi Qi Tang. Consists of: Huang Qi, Dang Shen, Dang Gui, Bai Zhu, Chen Pi, Sheng Ma, Chai Hu, Gan Cao, Sheng Jiang, Da Zao)	Hochu-ekki-to	Unexplained infertility.	Increase in motility and seminal plasma sFas levels correlated with concentration.	N/A
	3	Jeng et al 1997	Non-randomised controlled study	N/A	unknown	Shan Zhu Yu (Isolated substance of <i>Cornus officinalis</i> )	Aqueous extract prepared from dried fruit in phosphate buffered saline (pH 7.4)	28.5 % motility	Significantly improved motility. Increased to 42.8%.	N/A
varied: in vivo & in vitro	4	Crimmel et al 2001	Uncontrolled Case Reports	202 (in vivo); N/A in vitro	Twice/day for 60 days (in vivo); unknown (in vitro).	Lu Jiao Jia, Yin Yang Huo, Xian Mao, Jin Ying Zi, Sang Ji Sheng, Shi Di, Gou Qi Zi, Tu Si Zi, Shi Hu, Ba Ji Tian, Fu Pen Zi, Huai Niu Xi, San Leng, E Zhu (in vivo); 18 herbs (in vitro).	Sheng Jing (in vivo); several herbs un-named (in vitro).	Dg w poor SA.	Improvement in density, motility & grade. FSH, LH & T improved. Serum antibody titers reduced (in vivo); Huang Qi improved motility (in vitro).	78% pregnancy rate in available couples (in vivo).

Table 4. Methods and Results in Studies Evaluating Use of Chinese Herbal Medicine to Treat Male Infertility

**Methods and Results**

Articles		Study Design	n	Duration of Treatment	Single Herb or Herbal Extract	Herbal Formula	Semen Quality		Pregnancy Rate
							Before	After	
5	Yang et al 2003	Non-randomised controlled study	36	60 days	Fx consists of: Mo yao, Xiao hui xiang, Gan jiang, Yan hu suo, Dang gui, Chuan xiong, Rou gui, Chi shao, Pu huang, Wang bu liu xing, Xiang fu, Sheng shui zhi.	Shao Fu Zhu Yu Tang	Chronic Prostatitis Diagnosis with poor SA	Motility, Count & Morphology improved. Decrease in Free Radicals.	N/A
	Xian Xu et al 2003	Study Review (In-vitro and/or In-vivo)	N/A	N/A (several studies)	Lu Rong, Rou Gui, Du Zhong, Shan Zhu Yu, Gou Qi Zi, Bu Gu Zhi, Fu Pen Zi, Wu Wei Zi, Rou Cong Rong, Yin Yang Huo, Fu Ling, Ba Ji, Shu Di, Xian Mao, Shan Yao, Tu Si Zi, Che Qian Zi*	Study 1: Incubated (in vitro) in a solution of Xian Mao, Tu Si Zi & Bai Ji for 30 minutes. Study 2: 2 month non-rand, 202 men with Shengjing pill. Study 3: In vitro testing/trans-membrane migration method (Huang Qi). Study 4: Gui Zhi Fu Ling Wan for 3 months.	Poor SA, vericocle, many Diagnosis.	Overall marked improvement with in vivo formulas. Only in vitro improvement was with Study 1, and in Study 3, only herb that showed improvement was Huang Qi. Semen profile improved (GZFLW) and inc in density (71%) and motility (62%). Circulatory disorder was modulated in 40 of 50 men w/ vericocle.	N/A
6	Liu et al 2004	Non-randomised controlled study (In-vitro)	30	15, 60 & 180 minutes of incubation.	6 single herb aqueous solutions: Huang Qi (Astragalus membranaceus), Ci Wu Jia (Acanthopanax senticosus), Panax ginseng & Ophiopogon japonicus, P. ginseng & Aconitum carmichaeli, Salviae miltiorrhiae, Polyporus umbellatus polysaccharide.	N/A	Diagnosed with infertility	Huang Qi & Ci Wu Jia showed significant effect on motility in vitro.	N/A
7									
8	Tempest et al 2005	Non-randomised controlled study.	6	6 weeks- 6 months	**	N/A	Sperm Disomy	Significant reduction in disomy.	N/A

Table 4. Methods and Results in Studies Evaluating Use of Chinese Herbal Medicine to Treat Male Infertility

**Methods and Results**

Articles	Study Design	n	Duration of Treatment	Single Herb or Herbal Extract	Herbal Formula	Semen Quality		Pregnancy Rate
						Before	After	
9 Fu et al 2005	Randomised Study. (Combination Acup-Herbs)	100	unknown	Fx consists of: Shu Di Huang, Shan Zhu Yu, Mu Dan Pi, Shan Yao, Fu Ling, Ze Xie	Liu Wei Di Huang Wan	Dg Infertility & Elev AsAb	AsAb significantly decreased. 90% effectiveness in Acup-Herb group. 64% effectiveness in Prednisone group.	N/A
10 Akashi et al 2006	Prospective Randomized Study	20	3 months	Fx consists of Bu Zhong Yi Qi Tang (7.5g/day)	Bu-Zhong-Yi-Ki-Tang	Idiopathic oligozoospermia and/or asthenozoospermia (<20mL).	Motility increased, but Volume, Count & Morphology did not change.	N/A
11 Sun et al 2006	Prospective Randomized Study	200	unknown	Tao Ren, She Chuan Zi, Dang Gui, Nu Zhen Zi, Rao Cong Rong, Yi Zhi Ren, Shan Yu Rao, Yin Yang Huo, Wu Wei Zi, Gou Qi Zi, Gan Cao	Yikang Tang	Dg with Immune Infertility	Reduced AsAb & agglutination. Density, Motility & Pregnancy outcome increased. Improvement rate of symptoms & stability of therapeutic effect was more dramatic in herb group even after termination of therapy.	Increased
12 Tempest 2008	Double-blind placebo controlled clinical trial and assay. (In vitro assays)	N/A	N/A	Huang Bai, Nu Zhen Zi, Shan Yao, Shan Zhu Yu, Tu Si Zi, Ze Xie, Ba Ji Tian, Fu Pen Zi, Tao Ren, Hong Hua, Bai Zhu, Cang Zhu, Huang Qi, Jin Yin Hua, Chi Shao, C.Niu Xi, Fu Ling, Gou Qi Zi, Mu Dan Pi, Shu Di, Zhi Mu, Che Qian Zi, Dang Shen, Gan Cao, Wan Bu Liu Xing, Wu Wei Zi, Zhi Ke, Shi Chang Pu, Lian Zi Xin, Bi Xie, Chai Hu, Sheng Ma, Sheng Di, Chen Pi, Han Lian Cao, Yin Yang Huo, Yi Yi Ren	7 Decoctions were made from these 37 single herbs based on patient's previously used Male-Factor formula.	Hormone assays: investigated endocrine (anti-estrogen, specially) and antioxidant activity. Measured activity in single herbs and formulas. No human subjects used.	Some herbs increased estrogenic activity while others decreased. Some herbs were found to have strong hydroxyl scavenging activity.	N/A

Table 4. Methods and Results in Studies Evaluating Use of Chinese Herbal Medicine to Treat Male Infertility

**Methods and Results**

Articles	Study Design	n	Duration of Treatment	Single Herb or Herbal Extract	Herbal Formula	Semen Quality		Pregnancy Rate
						Before	After	
13 Huang & Chen 2008	Study Review	N/A	N/A	N/A	1 formula mentioned for treating male-immunological infertility was mentioned: Zhuanyindan + prednisone.	N/A	N/A	TCM showed an increase in pregnancy rates when used for hormone balance, IVF, implantation, ovulation, stress related infertility & immune infertility, both in men & women.
14 Ahmad et al 2010	Prospective study	150	3 months	Withania somnifera root powder 5g/day with milk.	N/A	Semen Analysis, Oxidative Biomarkers, Hormones measured in infertile men without infection or health issues, and with fertile female partners.	Inhibited lipid peroxidation & protein carbonyl content, and improved sperm count & motility. Increased diverse antioxidant enzymes and vit's A,C&E. Also corrected fructose. Increased serum T & LH and reduced FSH & PRL, congruent with good semen quality.	N/A
15 Geng et al 2010	Prospective Randomized Study	80	3 months	N/A	Tianxiong powder & Wuzi Yanzong pill	Oligospermia & Asthenospermia	Both formulas improved count & motility, and the Tianxiong powder also improved morphology.	N/A

\* These herbs were not used in one single study; rather, they were summarized in the review's analysis as the most common used in treating male infertility.

\*\* Red peony root (Chi Shao), Cyathula root, Poria (Fu Ling), Lyciu, fruit (Gou Qi Zi), Phellodendron bark (Huang Bai), Moutan (Mu Dan Pi), Ligustrum seed, Dioscorea root, Cornus fruit, Persica, Dadder seed, Alismatis rhizome, anemarrhena, morinda root, Alba atractylodes, Plantago seed, Codonopsis, Rubus, Liquorice root (Gan Cao), Safflower, Vaccaria seed, Schisandra fruit, Aurantium fruit, Acorus rhizome, Lotus embryo, Hypoglauca yam, Attractylodes, Bupleurum, Bugbane rhizome, Astragalus root (Huang Qi), Fresh rehmannia, Citrus peel (Chen Pi), Lonicera flower, Epimedium (Yin Yang Huo), Coix Seed (Yi Yi Ren).

 Review of several studies.

 Combination of acupuncture & Chinese herbal medicine used in 1 single study.