

Comparing the Effects of TCM Therapy Durations on Pregnancy Outcome of Infertile
Advanced Maternal Aged Women

A Capstone Project

Submitted in partial fulfillment of the requirements for the
Doctor of Acupuncture and Oriental Medicine Degree

By

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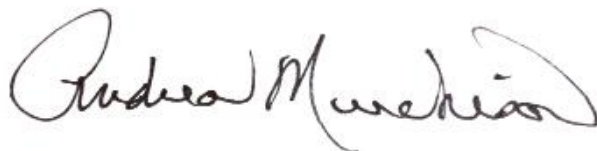
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ABSTRACT

Fertility declines with age, and age is one of the most important factors that affect female fertility. There is an increasing population of advanced maternal aged (AMA) women with infertility, however, their treatment options are often limited, and prognoses are poor. Traditional Chinese Medicine (TCM) has been utilized as a complementary and alternative medicine modality for treating infertility. The goal of this study was to investigate the effects of TCM treatment durations (less than 3 months vs. more than 3 months) on the pregnancy outcome (yes or no) of AMA women with infertility. A retrospective chart review of more than 500 charts using purposive, convenience sampling was engaged, and 67 eligible charts were reviewed. The results revealed that there was no significant difference between the two TCM treatment durations. From the findings of the current study, it is reasonable to state that AMA patients are likely to respond to TCM therapy within the first 3 months of treatments. Although not statistically significant, this trend from the current study implies that the 3-month period may be considered as an appropriate treatment cycle for TCM therapy when treating AMA patients with infertility. This finding may indicate the 3-month period treatment duration as an important milestone for TCM practitioners to assess patients' progress. However, when AMA patients continue with TCM treatments longer than 3 months, it appears to have no adverse effects on pregnancy outcomes, since pregnancy results were seen among some of the AMA patients studied in this review. The current study initiated a new area of research on determining an effective duration of TCM therapy to achieve pregnancy among infertile AMA patients.

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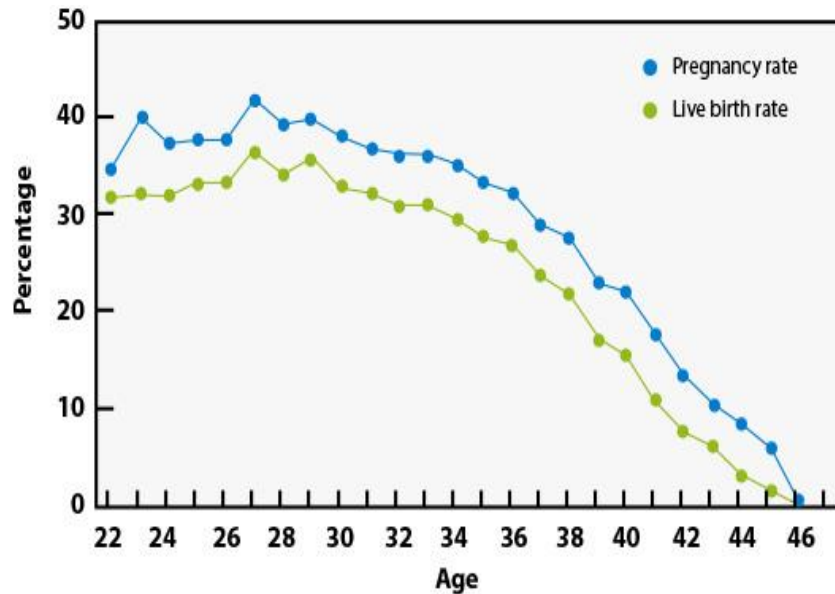
CHAPTER ONE: INTRODUCTION

Background

Age-related infertility as a medical problem is becoming more common in today's society. Approximately 1 in 5 women in the United States has her first child after age 35 according to the American Society of Reproductive Medicine (ASRM). Several factors have contributed to this trend, including improved contraception and delayed childbearing. Greater interest in advanced education and careers among women as well as later marriage and more frequent divorce are other factors that have contributed to this trend (Fritz & Speroff, 2011). According to the Center for Disease Control (CDC), more couples are experiencing infertility with higher prevalence among women over 34 years of age (CDC, 2008). Fertility declines with age, and age is one of the most important factors that affect female fertility (Fritz & Speroff, 2011). Advanced maternal aged (AMA) is defined as 35 years or older (Wang et al., 2011). Infertility for AMA women is defined as inability of a couple to conceive after 6 months of unprotected intercourse (U.S. Department of Health and Human Services, 2013). AMA women with infertility constitute the largest portion of the total infertility population (Sharara, Scott, & Seifer, 1998).

Naturally, there is an age-related decline in fecundity (George & Kamath, 2010). Female fertility peaks between ages of 20 and 24 and begins to decline more rapidly and progressively after late 20s and early 30s (Fritz & Speroff, 2011) with a dramatic fall after the age of 37 (George & Kamath, 2010). A chance of natural conception per month for a healthy 30-year-old woman is about 20% (ASRM, 2003), and it decreases to below 10% above the age of 35 (Spandorfer et al., 1998). By age 40, natural monthly fecundity is only about 5% per month (ASRM, 2003). In other words, the average time for AMA couple to conceive is 1-2 years

(American Pregnancy Association (APA), 2012) while approximately 85-90% of healthy young couples conceive within 6 months to 1 year (Fritz & Speroff, 2011).



ASRM Practise Committee. Aging and infertility in woman. *Fertil Steril* 2006.

Figure 1. Aging and Infertility in Women (ASRM Practice Committee, 2006).

Natural fecundity as well as assisted reproductive technology (ART) success is significantly lower for women in their late 30s and 40s except with egg donation (Reproductive Endocrinology and Infertility Committee et al., 2011). ART encompasses all techniques involving direct manipulation of oocytes outside the body for the purpose of reproduction (Fritz & Speroff, 2011). ART includes in vitro fertilization embryo-transfer (IVF-ET), gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), and frozen embryo transfer (FET) (SART, 2013). Intrauterine insemination (IUI) another procedure used for the purpose of assisting reproduction. IUI involves placing of sperm into a woman's uterus when she is ovulating. This procedure is used for couples with unexplained infertility, minimal male factor infertility, and women with cervical mucus problems. IUI is often done in conjunction with

ovulation-stimulating drugs, such as clomiphine citrate, gonadotropins, or urofollitropins (RESOLVE, 2013). Reported pregnancy rates per cycle for IUI range from 8 to 22% (Kamath et al., 2010). Fritz & Speroff (2011) state that age is the single most important factor affecting the probability of success with ART. One of the main factors leading to decreased fertility and poor ovarian response in ART is the age effect on oocytes (Fritz & Speroff, 2011; Surrey & Schoolcraft, 2000). In the 2011 national summary, the pregnancy rate per fresh embryo (from non-donor) transfer cycle was 46.2% for women under age 35, 38.5% for ages 35-37, 29.3% for ages 38-40, 19.5% for ages 41-42, and 9.1% for women aged over 42 years (SART, 2013). Similarly, the live birth rates per cycle in this summary was 40.1% for women under age 35, 31.9% for ages 35-37, 21.6% for ages 38-40, 12.2% for ages 41-42, and 4.2% for women aged over 42 years (SART, 2013). Furthermore, advanced maternal age is also associated with increased risks for pregnancy complications such as miscarriage, chromosomal abnormalities, hypertensive complications, pre-term birth, low birth weight infant, as well as stillbirth (Heffner, 2004).

This natural decline of female fertility potential is inevitable. This is due to the aging of the female reproductive system including the hypothalamic-pituitary-ovarian axis, the ovaries, as well as the uterus and oocyte depletion due to progressive atresia (Gindoff & Jewelewicz, 1986). Primordial follicles grow and undergo atresia from infancy to menopause. This process results in a decrease in the number of follicles from 6-7 million during gestation, 1-2 million at birth, and 300,000 to 500,000 follicles at menarche, and by age of 40, the follicular pool declines to approximately 25,000 and at menopause, less than 1,000 (Fritz & Speroff, 2011). Studies indicate the deleterious influence of AMA on oocyte yield and quality (Nichi et al., 2011). In fact, AMA women present a reduction in follicular diameter when compared to younger women, suggesting

that only larger follicles are generally recruited in the beginning of reproductive life, and as women get older the remaining follicles show a decrease not only in diameter but in quality as well (Nichi et al., 2011). The figure below illustrates the decline of follicles with advancing age.

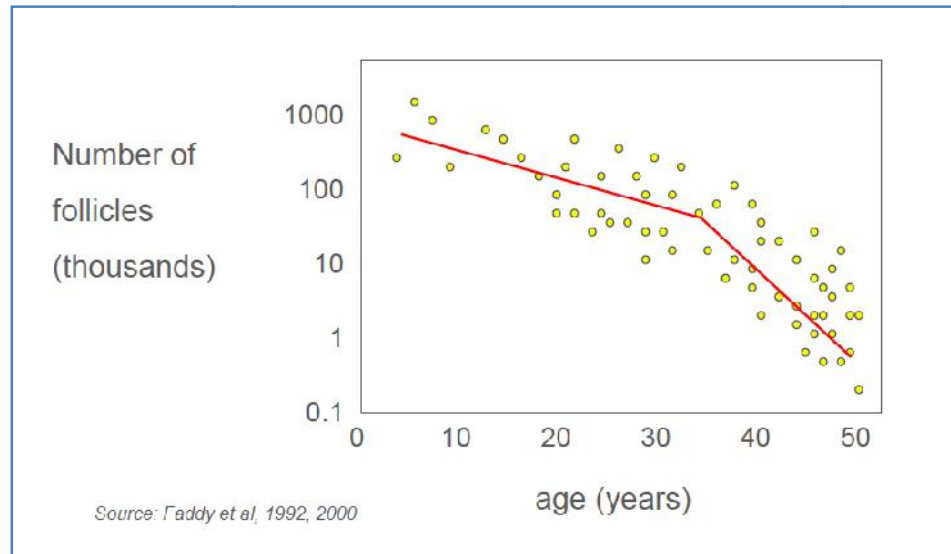


Figure 2. Number of follicles and advancing age

The clinical signs of follicular depletion include elevated follicular-stimulating hormone (FSH), decrease in inhibin B, and a shortened follicular phase due to an advanced follicular development (Fritz & Speroff, 2011). Diminished ovarian reserve (DOR) can be measured by biochemical tests such as FSH, estradiol, inhibin B, and antimullerian hormone (AMH) and ultrasonographic tests for antral follicle count and ovarian volume (Fritz & Speroff, 2011). An FSH level greater than 10 IU/L is associated with poor prognosis for ovarian stimulation response (Fritz & Speroff, 2011). An early elevation of serum estradiol (> 60-80 pg/mL) also reflects diminished ovarian reserve and a poor prognosis for stimulation response. An early elevation of estradiol also indicates advanced follicular development and early selection of a dominant follicle, and will suppress otherwise elevated FSH concentrations (Fritz & Speroff, 2011). The likelihood of pregnancy and stimulation response are poor when both FSH and estradiol are elevated (Fritz & Speroff, 2011).

Anti-mullerian hormone (AMH) is produced by the granulosa cells of preantral and small antral follicles, beginning when primordial follicles start development and ending when they reach diameter of 2-6 mm (Fritz & Speroff, 2011). The number of small antral follicles correlates with the size of the residual follicle pool and AMH levels decline progressively, becoming undetectable near the menopause (Fritz & Speroff, 2011). A lower level of serum AMH (0.2-0.7 ng/mL) as well as low antral follicle counts of less than 3 are considered as a predictor for poor response to ovarian stimulation for ART (Fritz & Speroff, 2011).

The currently available treatment options for AMA women with diminished ovarian reserve or infertility are limited other than egg donation (Reproductive Endocrinology and Infertility Committee et al., 2011). This limitation is due to their poor prognosis for pregnancy outcome with currently available reproductive assisting treatments such as IVF and controlled ovarian stimulation treatments (Reproductive Endocrinology and Infertility Committee et al., 2011). Therefore, there is a need for a research that investigates treatment options that can improve reproductive functions and increase pregnancy outcomes of infertile AMA women.

Complementary and alternative medicine (CAM) is widely used for the treatment of infertility (Weiss, Harris, & Smith, 2011). Acupuncture is the most commonly used CAM fertility treatment in the U.S. and has the most literature-based support (Weiss, Harris, & Smith, 2011). Similarly, herbal therapy is another CAM modality that infertile couples commonly seek and use (Smith et al., 2010). Acupuncture and Chinese herbal medicine (CHM) therapies are the main modalities of Traditional Chinese Medicine (TCM), which is an ancient medical system that is more than 2000 years old (Kaptchuk, 2000). Acupuncture involves insertion and manipulation of thin metallic needles into anatomically defined locations on the body to affect physiological functions. The traditional theory of acupuncture treatment is based on the premise

that there are patterns of energy flow (Qi) through the body that are essential for health. In TCM theory, diseases derive from disruption of this healthy energy flow as well as deficiencies and excess of organ systems. The traditional objective of acupuncture treatment is to correct such imbalances of energy flow by manipulating acupuncture points located throughout the body. Acupuncture points are chosen based on treatment principles that address individualized diagnoses. Similar to acupuncture, individualized herbal formulations are constructed based on a TCM diagnosis determined by symptom presentations and pattern differentiations.

Infertility in TCM

The earliest treatments of infertility was recorded in Sun Si Miao's book *Thousand Golden Ducat Prescriptions (Qian Jin Yao Fang, 652A.D)* and later in the Song dynasty (960-1279), in the *Great Treatise of Useful Prescriptions for Women (Fu Ren Liang Fang Da Quan, 1237)* (Maciocia, 2011). From the perspective of TCM theory, common differential diagnostic patterns for infertility include Kidney deficiency, Blood deficiency, Blood stasis, Cold in the Uterus, and Damp-Phlegm (Maciocia, 2004). Kidney system is the one of the main organ systems that governs reproductive function (Maciocia, 2004). The Kidney essence is the basis for the *Tian Gui*, which is equivalent of hormonal system including reproductive organs and their functions in modern medicine (Maciocia, 2011). As an overall pattern, Kidney deficiency has been found to be the most common diagnosis among infertility (Maciocia, 2011). One of the infertility pattern associated with advanced maternal age is the declining of the *Tian Kui* and the Kidney Essence. In fact, female physiology and its natural decline were described in one of the early ancient treatises, the *Yellow Emperor's classics of medicine (Huangdi Neijing)* from around 2600 BC. It is mentioned that secondary sex characteristics are complete at age 28, representing

the height of female development, and at age 35, women's Qi and Blood begin to decline where yangming channels start to deplete (Ni, 1995).

TCM therapy has been utilized from the ancient time to the modern time as a medical modality to treat infertility in China. Today, TCM has been utilized as a sole or in conjunction with western reproductive assisting treatments such as ART in the U.S. as well as in China. Acupuncture has been demonstrated to effectively normalize ovulation (Song, Zheng, & Ma, 2008; Yang et al., 2005; Stener-Victorin et al., 2000; Chen, 1997), and regulate the hypothalamic-pituitary-ovarian (HPO) functions and gonadotropin secretions (Huang & Chen, 2008; Mo et al., 1993; Chen, 1997; Yu, Zheng, & Ping, 1989). Acupuncture has also been demonstrated to improve pregnancy outcomes of IVF (Paulus, 2002; Westergaard, 2006; Dierterle et al., 2006; Magarelli, Cridennda, & Cohen, 2009; Zheng et al., 2012). Similarly, herbal therapy is another CAM modality that infertile couples commonly seek and use (Smith et al., 2010). Chinese herbal medicine (CHM) has also been demonstrated to improve pregnancy outcomes (Wing, 2009; Wing & Sedlmeier, 2006; Reid & Stuart, 2011). Rubin (2010) and Chen (2011) have also demonstrated that CHM can improve the pregnancy outcome of IVF.

TCM has been demonstrated to be an effective treatment modality for improving pregnancy outcomes and treating infertility associated conditions. However, despite the increasing popularity of the use of acupuncture and herbal therapy for reproductive assistance, only a few reviews have evaluated the quality of evidence underlying the use of CAM fertility treatments (Weiss, Harris, & Smith, 2011). The amount of literature available that demonstrate the effectiveness and mechanisms of acupuncture and CHM for treating infertility is still limited. Furthermore, to my best knowledge there is only a very few articles available that demonstrate effective treatments for improving the pregnancy outcomes of age related infertility. There is a

need for more research to be added to currently existing knowledge on the efficacy of TCM therapy, effective method of administration as an established TCM treatment cycle as well as safety. Furthermore, a new area of research on the effectiveness of TCM therapy for age related infertility is needed because of the increasing patient population, limited treatment options as well as poor prognosis for this patient population.

Research Objectives and Hypothesis

The purpose of this study is to compare the effects of different TCM therapy durations on the pregnancy outcomes of infertile AMA women. To my best knowledge, there is no literature available that demonstrates the most effective durations of TCM therapy for treating age related infertility. The benefits and efficacy of TCM therapy for treating infertility or related conditions have been studied and reported by various investigators mentioned above. Therefore, this study is intended to add a new knowledge about effective TCM treatment duration for treating infertility to the existing literatures. Secondly, this study is also intended to establish a new area of knowledge on TCM therapy for treating age related infertility. Furthermore, as an integration of TCM therapy and Western medical procedures become more common in the area of Reproductive medicine, it is important to define what a TCM treatment cycle is that can yield to its intended effect. With this knowledge established, Western medical professionals can better understand about TCM therapy. The knowledge from this study is intended to contribute for better integration and collaboration between TCM practitioners and Western medical professionals.

In my clinical experience, I have observed pregnancy and live birth successes achieved among infertile AMA women with using TCM therapy. Patients were able to conceive during TCM therapy as a sole therapy or used along with Western medical fertility treatments such as

ART. One of the most common questions presented by AMA patients to me was about the durations of TCM therapy that yields to pregnancy. Often there seems to be a dilemma that AMA patients struggling with infertility face; two stressful factors that one, pregnancy may take a long period of time to achieve and two, the fact of declining reproductive physiology. On top of having stress around infertility, AMA patients often feel stressed especially when it comes to spending months for treatments. This is because they feel that their reproductive function and chances of conceiving are declining as time passes. I was unable to provide an evidence based answer to this question other than providing them with recommendation of 3-6 months based on available evidence. Among available literature, the most common duration of TCM therapy used or TCM therapy demonstrating its efficacy in improving pregnancy outcome appears to be 3 months or more. Wing & Sedlmeier (2006) observed that with CHM therapy, most pregnancies occur in the first six cycles and the pregnancy rate drops dramatically after six cycles, implying that CHM attains its maximum effects in the first six treatment cycles. A meta-analysis by Reid & Stuart (2011) found improved pregnancy outcome in a 4-month period from CHM treatment. For acupuncture, treatments over 3 menstrual cycles yielded to a higher pregnancy rate among women in acupuncture group compared to the women in clomiphene group (Song, Zheng, & Ma, 2008). From this observation of the currently available literature, TCM treatment duration of more than 3 month appears to be an effective duration yielding to intended outcomes.

From Western physiological point of view, ovarian folliculogenesis involves a steady process of multi-faceted follicular recruitment. Cohorts of resting primordial, also called non-growing follicles (NGFs), are consistently recruited though, ultimately, only one single oocyte usually reaches ovulation (Coccia & Rizzello, 2008). Although how primordial follicles are activated to enter maturation is not well understood, the process is speculated to involve complex

bi-directional signalling between oocytes and surrounding somatic cells (McLaughlin & McIver, 2009). The entire period of ovarian folliculogenesis, including the transition of primordial follicles into pre-ovulatory follicles is estimated to be more than 175 days (Gougeon, 1986). The time that elapses in progressing from a primary follicle to ovulation is approximately 85 days (Gougeon, 1986; Gougeon, 1996; Macklon & Fauser, 1999). The figure below illustrates the process of folliculogenesis.

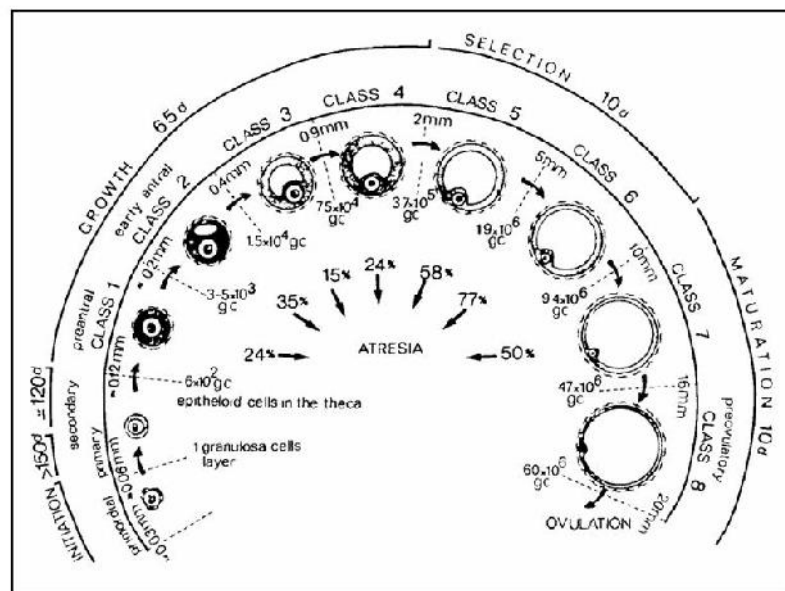


Figure 3. Folliculogenesis (Gougeon, 1986)

The exact mechanisms and effects of acupuncture and CHM on the folliculogenesis have not been studied. However, based on the fact that acupuncture and CHM can improve various physiological mechanisms involved in reproductive physiology, it is reasonable to speculate the potential benefits of TCM therapy on follicles at the antral, pre-antral, or primordial stage of folliculogenesis. This, in turn, may contribute to improve the growth and selection process of the oocyte, leading to better pregnancy outcome. Determining the effects of the TCM therapy on the

folliculogenesis is not within the scope of this study. However, based on the observations from literature on TCM therapy for treating infertility and related conditions as well as the mechanisms involved in folliculogenesis, I hypothesize that TCM treatment duration of more than 3 months would yield to better pregnancy outcome compared to TCM treatment duration of less than 3 months.

This project will proceed with the literature review in chapter two followed by the explication of the research method in chapter three. Consequently, chapter four will include the results and analysis followed by chapter five wherein discussion regarding the findings of the current study will be engaged.

DEFINITIONS OF TERMS

ABBREVIATIONS:

- **AFC:** Antral follicle count
- **AMA:** Advanced maternal age
- **AMH:** Antimullerian hormone
- **ART:** Assisted Reproductive Technologies
- **COS:** Controlled ovarian stimulation
- **CHM:** Chinese Herbal Medicine
- **DOR:** Diminished ovarian reserve
- **E2:** Estradiol
- **FET:** Frozen embryo transfer
- **FSH:** Follicular-stimulating hormone
- **HPOA:** Hypothalamic-Pituitary-Ovarian-Axis
- **ICSI:** Intracytoplasmic sperm injection
- **IVF:** In vitro fertilization
- **IUI:** Intrauterine Insemination
- **LH:** Leutinizing hormone
- **FSH:** Follicular stimulating hormone
- **FET:** Fronzen Embryo Transfer
- **OI:** Ovarian induction
- **P:** Progesterone
- **PCOS:** Polycystic ovarian syndrome
- **TCM:** Traditional Chinese Medicine

DEFINITIONS

- **Antral follicle count (AFC):** The total number of antral follicles measuring 2-10 mm in both ovaries during the early follicular phase and is a useful measure of ovarian reserve as it quantifies the number of follicles at the stage of development that responds to ovarian stimulation (Fritz & Speroff, 2011).
- **Advanced maternal age (AMA):** 35 years and older (Wang et al., 2011)
- **Assisted Reproductive Technologies (ART):** All techniques involving direct manipulation of oocytes outside of the body such as IVF, GIFT, ZIFT, TET, ICSI, and PGD (Fritz & Speroff, 2011).
- **Controlled ovarian stimulation (COS):** A medical reproductive assisting treatment that involves the use of exogenous gonadotropins to stimulate the development and ovulation of more than one mature ovum in efforts to increase cycle fecundity. This procedure is often combined with timely IUI (Fritz & Speroff, 2011).
- **Diminished ovarian reserve (DOR):** A threshold value above which is associated with a reduction in the oocyte pool, leading to impaired fertility (Scott et al., 1989). DOR is associated with increased subfertility, miscarriage rates, and poor ovarian response, higher cancellation rates, and reduced live birth rates with assisted reproduction (Sun et al., 2008). DOR can be measured by biochemical tests such as FSH, estradiol, inhibin B, and AMH and ultrasonographic tests for antral follicle count and ovarian volume (Fritz & Speroff, 2011). An FSH level greater than 10 IU/L is associated with poor prognosis for ovarian stimulation response (Fritz & Speroff, 2011). An early elevation of serum estradiol (> 60-80 pg/mL) also reflects diminished ovarian reserve and a poor prognosis for stimulation response. Lower level of serum AMH (0.2-0.7 ng/mL) as well as low antral

follicle counts of less than 3 is considered as a predictor for poor response to ovarian stimulation for ART (Fritz & Speroff, 2011).

- **Electroacupuncture:** A variation of traditional acupuncture. In this process, acupuncture needles are placed at selected points and then pulsed with an electric current to stimulate the acupuncture points (NCCAM, 2012).
- **Fecundity:** The probability that a cycle will result in a live birth (Fritz & Speroff, 2011).
- **Intracytoplasmic sperm injection (ICSI):** A technique used as a form of ART indicated especially for male factor infertility. A single selected sperm is first immobilized by compressing the sperm tail with an injection pipette, then drawn into the pipette, which then pierces the zona and oolemma and injected into oocyte cytoplasm (Fritz & Speroff, 2011)
- **Intrauterine insemination (IUI):** An artificial insemination performed by depositing sperm into the uterus to improve cycle fecundity (Fritz & Speroff, 2011).
- **In vitro fertilization (IVF):** Assisted reproductive technology involving a sequence of highly coordinated steps beginning with controlled ovarian hyper-stimulation with exogenous gonadotropins, followed by retrieval of oocytes from the ovaries, fertilization in the laboratory, and transcervical transfer of embryos into the uterus (Fritz & Speroff, 2011).
- **Moxibustion:** A TCM modality often used as an adjunctive therapy and involves application of heat near the skin at acupuncture point from the burning of the herb, moxa. Its intended therapeutic effect is to stimulate energy flow (NCCAM, 2012).

CHAPTER TWO: LITERATURE REVIEW

Overview

This chapter provides the scholarly foundation for the current study. A summary and review of the literature on the topic of TCM therapy (Acupuncture and Chinese herbal medicine) and its efficacy as a fertility assisting treatment as well as for treating reproductive related conditions are presented.

Currently available literature has demonstrated the efficacy of TCM modalities such as acupuncture and Chinese herbal medicine (CHM) for treating infertility or improving reproductive functions. However, there are only a few articles available that specifically study the area of advanced maternal age (AMA) related infertility or articles that demonstrate and define what a treatment cycle is for TCM therapy that yields its intended outcome. This chapter will first engage in the literature review of acupuncture followed by the review of literature on the integration of TCM therapy with Assisted Reproductive Technologies (ART). Thirdly, the review of literature related with CHM is presented. Lastly, literatures related with AMA related infertility and TCM treatments are presented.

Resources Engaged

Searches for this literature review included a web search in Pubmed, Google Scholar, Cochrane Review, Fertility and Sterility, Human Reproduction, Journal of Chinese Medicine, Chinese Journal of Integrative Medicine. Key search words included: acupuncture and ovulation, acupuncture and infertility, Chinese herbal medicine, Chinese herbs, and Chinese medicine and fertility, acupuncture and fertility, Chinese herbal medicine and fertility, acupuncture and pregnancy, Chinese herbs and pregnancy.

Acupuncture for Fertility

Acupuncture has become a popular treatment choice for infertility (Smith et al., 2010). One of the evidence based scientific mechanisms of acupuncture, it has been found that the acupuncture points demonstrate higher electrical conductance due to the presence of higher density of gap junctions along cell borders (Chang, Chung, & Rosenwaks, 2002). Moreover, acupuncture points act as converging points for electromagnetic fields, and a higher metabolic rate, temperature, and calcium ion concentration have also been observed at these points (Chang, Chung, & Rosenwaks, 2002). In the area of reproductive medicine, acupuncture has been shown to benefit reproductive functions and pregnancy outcomes by modulating endogenous regulatory systems such as the sympathetic nervous system, endocrine system, and the neuroendocrine system and also by improving the uterine and ovarian blood flow and endometrial receptivity.

Effects of Acupuncture on Neuroendocrine and Endocrine Functions

Acupuncture has been shown to cause a significant increase in beta-endorphin levels during treatment (Petti, et al., 1998). Opioid peptides such as beta-endorphin have been demonstrated to play an important role in the neuroendocrine aspect of a healthy reproductive system. The increase in beta-endorphin levels in the brain, in turn, affects the release of gonadotropin releasing hormone (GnRH) by the hypothalamus, follicle-stimulating hormone (FSH) from the pituitary gland, and estrogen and progesterone levels from the ovary (Ng, 2008; Huang, 2008; Lim 2010; Stener-Victorin, 2010). This intricate cascade of neuroendocrine and endocrine events constitutes a healthy hypothalamic-pituitary-ovarian axis (HPOA) mechanism that is essential for healthy reproductive function. The role of opioid peptides such as beta-endorphin in the initiation of the mid-cycle LH surge for ovulation in normal cycling women was also demonstrated by Rossmanith et al. (1988). Furthermore, beta-endorphin has also been

found to have a peripheral impact on reproductive physiology. Petraglia et al. (1987) and Petraglia et al. (1985) found much higher beta-endorphin levels in ovarian follicular fluid of healthy ovulatory women than in their circulating plasma. The highest level of beta-endorphin was also found to be in the preovulatory follicle (Petraglia et al., 1987). In comparison, the concentration of beta-endorphin was found to be undetectable in ovarian follicular fluids among postmenopausal women (Petraglia et al., 1985). From these findings, beta-endorphins seem to play an active role in normal reproductive physiology such as ovulation, regulation of HPO axis, and hormonal secretions. From these findings, the effect of acupuncture on beta-endorphin appears to be one of the critical mechanisms that acupuncture can benefit reproductive functions.

Various investigators conducted studies and confirmed that acupuncture can effectively normalize ovulation (Song, Zheng, & Ma, 2008; Yang et al., 2005; Stener-Victorin et al., 2000; Chen, 1997). Furthermore, acupuncture has been shown to regulate the HPO functions and gonadotropin secretions (Huang & Chen, 2008; Mo et al., 1993; Chen, 1997; Yu, Zheng, & Ping, 1989). Earlier literature involving small animal study also established this finding of the effect of acupuncture on restoring normal ovulation and regulating HPO axis and gonadotropin secretions (Lin et al, 1988; Chen, 1993; Yang, Yu, & He, 1994). Cai (1997) conducted a cohort, observational clinical study (n=11) and demonstrated that acupuncture can be used as a surrogate for hCG in ovulation induction and prevent chances of ovarian hyper-stimulation syndrome (OHSS). Gerhard and Posteck (1992) found that auricular-acupuncture to be an effective fertility therapy for women with hormonal disorders such as oligomenorrhea and luteal deficiency. 90 women were included in this study and were separated into the acupuncture group and the control group who received hormone treatments. The pregnancy rates were similar in both

groups, indicating that acupuncture may be as effective as hormone therapy to improve reproductive functions and therefore yielding to pregnancy.

Acupuncture not only has stimulatory effects on the HPO axis and gonadotropin secretions, acupuncture also has regulating and normalizing effects on these mechanisms by its ability to inhibit hyper-secretion of gonadotropins (Chen, 1997) via central sympathetic inhibition (Ng, et al., 2008). Aso et al. (1976) also confirmed that the rise of hormone levels such as LH, FSH, estradiol, and progesterone by acupuncture were all within the normal range when administered on normal ovulatory women. These findings further confirm the regulatory effects of acupuncture on HPO axis and gonadotropin and hormonal secretion. Furthermore, it is reasonable to state that acupuncture is safe without risks of hyper-stimulation of HPO axis and serum hormone levels.

Effects of acupuncture on improving pregnancy outcomes were also explored by several investigators. Song, Zheng, and Ma (2008) conducted a randomized controlled trial with 120 infertile women with ovulatory dysfunction. The trial compared the effects of acupuncture (plus moxibustion) to medication (oral clomiphene citrate) After treatment of three menstrual cycles, women in both group showed similar increase in ovulation rates, however, acupuncture group resulted in significantly higher pregnancy rate ($P < 0.05$). Similarly, an earlier randomized controlled trial by Yang et al. (2005) also found a significantly higher pregnancy rate ($P < 0.05$) among acupuncture group (65% pregnancy rate) compared to clomiphene group (45% pregnancy rate). This study involved 240 women with endocrine dysfunctional infertility and 160 women receiving acupuncture and 80 women receiving clomiphene citrate treatment.

Acupuncture points used in the literature reviewed were similar, including the most common points used; Ren-4 (Guanyuan), Ren-3 (Zhongji), and SP-6 (Sanyinjiao) (Chen, 1997;

Cai, 1997; Mo et al., 1993; Yu, Zheng & Ping, 1989). Other points also used were Zi Gong (EX CA-1) (Chen, 1997; Cai, 1997; Song, Zheng, & Ma, 2008), and UB-23 (Shenshu) (Cai, 1997; Mo et al.,1993) and UB-18 (Ganshu), and ST-36 (Zusanli) (Song, Zheng, & Ma, 2008). Stener-Victorin et al. (2000) used UB-23 (Shenshu), UB-28 (Pangguangshu), SP-6 (Sanyinjiao), SP-9 (Yinlingquan), PC-6 (Neiguan), TB-5 (Waiguan), and GB-20 (Feng Chi) in their treatment protocols. Song, Zheng, and Ma (2008) also utilized suspended moxibustion at Ren-8 (Shenque) and SP-6 (Sanyinjiao). Electro-acupuncture was commonly used among the available studies (Chen, 1997; Yu, Zheng and Ping, 1989; Stener-Victorin et al., 2000).

Effects of Acupuncture on Uterine and Ovarian Blood Flow and Uterine Receptivity

Acupuncture has been demonstrated to impact reproductive functions by improving uterine blood flow, ovarian blood flow, and uterine morphology and receptivity (Stener-Victorin et al.,1996; Huang, 2008; Jin et al., 2009; Fu et al., 2010). One of the mechanisms that acupuncture involves is inhibition of sympathetic activity, which in turn improves blood flow to the ovaries and the uterus (Stener-Victorin et al.,1996; Stener-Victorin et al., 2004; Lim & Wong, 2010).

Endometrial thickness, endometrial morphology, and uterine artery blood flow have been implicated as important parameters for successful embryo implantation and pregnancy outcome (Chiang et al., 2000; Giudice,1999; Chien et al., 2002; Al-Ghamdi et al., 2008; Momeni et al., 2011). Giudice (1999) demonstrated endometrial receptivity and normal endometrial morphology is one of the important maternal physiological factors affecting fertility. An earlier study by Lessey et al. (1995) found reduced endometrial receptivity among an increasing number of unexplained infertilities. Steer et al. (1994) also concluded from their findings that the impedance of the uterine blood flow may be an important contributing factor to infertility.

Furthermore, similar to the aging effects of the ovaries, the uterus also undergoes its aging effect, including reduced vascular perfusion (Goswamy, Williams, & Steptoe, 1988). Chiang et al. (2000) found that increased uterine perfusion in the early follicular phase enhanced the pregnancy rate of IVF in women aged 40 and above. The authors of this study stated that AMA patients, particularly aged 40 or older with poor basal uterine perfusion, should be identified early in the early follicular phase of the menstrual cycle and apply appropriate intervention to improve the uterine circulation for the subsequent chance of pregnancy (Chiang et al., 2000).

Chien et al. (2002) also investigated the effect of endometrial receptivity on pregnancy and implantation rates by using transvaginal color Doppler examination. The authors found that the presence of both endometrial and subendometrial blood flow is indicative of good endometrial receptivity while the absence of both represents a poor uterine environment (Chien et al., 2002). Consequently, the pregnancy and implantation rates of patients with the presence of both endometrial and sub-endometrial flow were found to be 5.9 times higher than those with the undetectable endometrial and sub-endometrial flow (Chien et al., 2002). Al-Ghamdi et al. (2008) also found a positive correlation between endometrial thickness and pregnancy rate in IVF. A meta-analysis of 14 studies by Momeni et al., (2011) further confirmed earlier findings of the positive, significant correlation between higher endometrial thickness and higher pregnancy rates from IVF. Stener-Victorin et al. (1996) found that electro-acupuncture is effective in improving uterine blood flow by reducing uterine blood impedance via inhibition of sympathetic nervous system. This study was a prospective, non-randomized study, involving 10 infertile women aged between 25-40 years old. A total of 8 acupuncture treatments were giving over 4-week period, and pulsatility index (PI) was measured as an indicator of uterine blood flow.

Ho et al. (2009) further investigated the effects of electro-acupuncture on uterine blood flow and also pregnancy rate. This study was a prospective, randomized study involving 44 women undergoing IVF. Electro-acupuncture was given twice a week for 2 weeks, a total of 4 treatments before the oocyte retrieval. Pulsatility index (PI) indicating uterine blood flow and pregnancy rates were measured. The authors of this study found a significant reduction in PI, indicating improved uterine blood flow in the EA group; however, there was no significant increase found in pregnancy rate in this study. Jin et al. (2009) conducted an animal study and found positive effects of acupuncture on endometrial morphology.

Uterine contraction is another mechanism that plays an important role in reproductive physiology. Stress stimulates the sympathetic nervous system, which may indirectly cause constriction of ovarian and uterine arteries as well as contraction of the uterus. Similarly, during an embryo transfer, uterine contraction can be initiated by the stimulation of the cervix by the catheter (Mansour & Aboulghar, 2002). Stimulation of the cervix causes release of oxytocin, which in turn increases uterine contractility (Mansour & Aboulghar, 2002). Prevention of the uterine contraction has been recommended in order to support embryo implantation by preventing an immediate or delayed expulsion of the embryos (Mansour & Aboulghar, 2002). Although adequate uterine contractility may be required for successful embryo implantation by providing for gamete/embryo transportation through the utero-tubal cavities and avoiding ectopic pregnancies (Bulletti et al., 2004; Bulletti & De Ziegler, 2006), uterine contraction during the time of implantation has been found to have negative effects on pregnancy outcome (Fachin et al., 1998; Mansour & Aboulghar, 2002). In fact, poorer pregnancy and implantation rates were seen among IVF patients displaying higher uterine contractility frequency compared with those presenting lower uterine contractility frequency (Fachin et al., 1998). Acupuncture has been

found to reduce uterine contractility and motility (Kim, Shin, & Na, 2000; Stener-Victorin et al., 1996). Kim, Shin, and Na (2000) conducted an animal study and suggested that LI-4 (Hegu) acupuncture treatment could be useful in inhibiting the uterus motility. In their rat experiments, acupuncture on LI-4 suppressed the expression of COX-2 enzyme in the endometrium and myometrium of pregnant and non-pregnant uteri. The reduction of uterus motility was observed during the acupuncture treatment.

Intraovarian blood flow is also an important physiological change that affects fertility (Yokota et al., 2000; Nakagawa et al., 2004; Kurjak et al., 1991). In fact, the reduction in intraovarian arterial pulsatility index (PI) value has been reported as a necessary condition for the production of a good quality oocyte (Nakagawa et al., 2004). Furthermore, a reduction of intraovarian arterial blood flow resistance appears to be necessary for achieving successful pregnancy in natural cycles (Nakagawa et al., 2004). Various animal studies also reported the effects of acupuncture on ovarian blood flow (Stener-Victorin, Kobayashi, & Kurosawa, 2003; Stener-Victorin et al., 2004)

Stener-Victorin, Kobayashi, & Kurosawa (2003) investigated changes in ovarian blood flow (OBF) in response to electro-acupuncture (EA) stimulation at different frequencies and intensities in anesthetized rats. Two frequencies-2 Hz (low) and 80 Hz (high) with three different intensities (1.5, 3, and 6 mA) were applied and compared. Low-frequency EA at 3 and 6 mA elicited significant increases in OBF. In contrast, high-frequency EA with an intensity of 6 mA evoked significant decreases in OBF. The authors of this study concluded that low-frequency EA stimulation of 2Hz is effective in increasing OBF as a reflex response via the ovarian sympathetic nerves.

Many of the earlier articles above demonstrating the mechanisms of acupuncture on physiological mechanisms associated with reproduction such as regulation of HPO axis and hormonal secretions as well as uterine and ovarian blood flow have provided scientific foundations for the following studies investigating the effects of acupuncture with ART.

Acupuncture and ART

Acupuncture has also been found to be an effective adjunctive therapy to improve pregnancy outcomes of ART. Paulus et al. (2002), Westergaard et al. (2006), Dierterle et al., (2006), Magarelli, Cridennda, & Cohen (2009), and Zheng et al. (2012) all found that acupuncture therapy improve clinical pregnancy rate of IVF and identified an effective method of acupuncture therapy administration when used in conjunction with IVF to improve clinical pregnancy rate of IVF.

Paulus et al. (2002)

Paulus et al. (2002) conducted a prospective randomized study and evaluated the effects of acupuncture on the pregnancy rate in IVF, involving 160 women with the average age of 32.5 and the age range of women between 28.5 and 36.5. The result of this study indicated that acupuncture is effective in improving pregnancy outcome of IVF. The investigators of this study randomized 160 patients to either receive either acupuncture (n=80) 25 minutes before and after embryo transfer (ET) or no acupuncture (n=80) but 25 minutes bed rest after ET as a control. Acupuncture treatment given before ET include P-6 (Neiguan), SP-8 (Diji), Liv-3 (Taichong), Du-20 (Baihui), and ST-29 (Guilai), ear points Shenmen and Brain on one ear, and Uterus and Brain in the other ear for 25 minutes before ET. The acupuncture treatment after ET included ST-36 (Zusanli), SP-6 (Sanyinjiao), SP-10 (Xuehai), and LI-4 (Hegu) with the ear points Shenmen and Brain on one ear and Uterus and Brain in the other ear but switched to the opposite

side from before ET. The acupuncture points were chosen to improve blood perfusion and more energy to the uterus (Paulus et al., 2002). The authors of this study found that the pregnancy rate for the acupuncture group is considerably higher than for the control group (42.5% vs 26.3%; $p=0.03$) and concluded acupuncture to be useful tool for improving pregnancy rate of ART. This study became a pioneer of the studies on acupuncture and IVF and established a foundation for the following acupuncture research investigating the effects on ART outcome.

Westergaard et al. (2006)

Westergaard et al. (2006) conducted a prospective randomized study involving 273 women and investigated the effect of acupuncture on ART pregnancy outcome. The age range of this study was 24-45 years old, and the average age in this study was age 37, which was older than the Paulus et al. (2002) study. The study design followed the Paulus et al. (2002) with a few changes. Westergaard et al (2006) included another acupuncture treatment group with an additional treatment at 2 days after ET. The women in this study were randomized into three groups; 1) No acupuncture (control group) (n=87), 2) Acupuncture on the day of ET (ACU 1 group) (n=95), and 3) Acupuncture as ACU 1 plus acupuncture again 2 days later (ACU 2 group) (n=91). Same acupuncture point protocol was used for before ET and after ET in this study as Paulus et al. (2002) (see above) minus the ear acupuncture points. Acupuncture treatment 2 days after ET include Du-20 (Baihui), Ren-3 (Zhongji), ST-29 (Guilai), SP-10 (Xuehai), SP-6 (Sanyinjiao), ST-36 (Zusanli), and LI-4 (Hegu). These points were chosen for relaxation and improvement of uterine blood perfusion to further enhance endometrial receptivity for implantation. The result of this study revealed clinical and ongoing pregnancy rates were significantly higher in acupuncture group (ACU1) than the control group (39% vs. 26% and 36% vs. 22%). The clinical and ongoing pregnancy rates in the ACU2 group (36% and 26%) were

higher than the control but the difference did not reach statistical difference. The authors of this study concluded that acupuncture on the day of ET significantly improve the reproductive outcome of IVF/ICSI, compare with no acupuncture and that repeating acupuncture on 2 days after ET provided no additional beneficial effects.

Dieterle et al. (2006)

Dieterle et al. (2006) also investigated the effects of acupuncture on pregnancy outcome of IVF/ICSI. The findings of this study supported the result of the earlier study by Paulus et al. (2002) and found that acupuncture has a positive effect on the outcome of IVF/ICSI. This study was a randomized, prospective, controlled clinical study involving 225 infertile patients undergoing IVF/ICSI. The age range of patients in this study was between ages 30-38. Patients were randomized into two acupuncture groups. Both groups received acupuncture treatments immediately after ET for 30 minutes as well as 3 days later of ET. Group I received acupuncture treatment with acupuncture points intended to support fertility according to TCM theory. The acupuncture points after ET for Group I include Ren-4 (Guanyuan), Ren-6 (Qihai), ST-29 (Guilai), P-6 (Neiguan), SP-10 (Xuehai), and SP-8 (Diji). Ear seeds were placed on ear point 55 (Shenmen), ear point 58 (Zigong), ear point 22 (Neifenmi), and on ear point 33 (Pi Zhi Xia). These seeds remained in place for 2 days and were pressed twice daily for 10 minutes. 3 days after ET, the acupuncture treatment given for Group I include Hegu (LI-4), Sanyinjiao (SP-6), Zusanli (ST-36), TaiXi (KD-3), and Taichong (Liv-3). In addition, the same ear points were pressed at the opposite ear twice daily, and the seeds were removed after 2 days. Group II received a placebo acupuncture treatment which was designed not to support fertility. The use of acupuncture protocol that is not intended to support fertility as a control group was the unique characteristic of this study, which differed from Paulus et al. (2002) and Westergaard et al.

(2006). The acupuncture treatment was given after ET and again 3 days after ET, including SJ-9 (Sidu), SJ-12 (Xiaoluo), GB-31(Fengshi), GB-32 (Zhongdu), and GB-34 (Yanglinggua) as well as ear points such as ear point 17 (Shangzhi), ear point 14 (Fengsi), ear point 8 (Sisheng), and ear point 53 (Jian). The results of this study found that Group I had significantly higher clinical and ongoing pregnancy rates (33.6% and 28.4% respectively) than Group II (15.6% and 13.8% respectively). The authors of this study concluded that luteal phase acupuncture has a positive effect on the outcome of IVF/ICSI. This study confirmed the findings of Paulus et al. (2002) and Westergaard et al. (2006), and further demonstrated the efficacy of acupuncture protocol that is designed to support fertility compared to the protocol that is unrelated to supporting fertility.

Zheng et al. (2012)

The positive effect of acupuncture on the pregnancy outcome of IVF was also confirmed by the most recent meta-analysis by Zheng et al. (2012). In this meta-analysis, twenty-three trials (a total of 5598 participants) were analyzed. The authors of this literature concluded that acupuncture, especially around the time of the controlled ovarian hyper-stimulation, improves pregnancy outcomes in women undergoing IVF. Zheng et al (2012) also recommended that more positive effects from acupuncture in IVF can be expected if a more individualized acupuncture programs are used.

Contrary to the above findings, prior to this meta-analysis, systematic reviews and meta-analyses of randomized controlled trials (RCTs) have lead to contradictory conclusions. The first two meta-analyses were performed by Manheimer et al. (2008) (7 trials with 1,366 participants) and Ng et al. (2008) (10 trials with 2,003 subjects) and reported that acupuncture given around ET significantly improved the clinical pregnancy rates of IVF. Manheimer et al. (2008) further concluded that acupuncture given around ET also improved ongoing pregnancy and live birth

outcome of IVF. However, the third analysis published by Cheong et al. (2008) (13 trials and 2,300 participants) concluded that acupuncture performed on the day of ET increases live birth rates but does not increase clinical pregnancy rates, and that there are no beneficial effects on pregnancy outcomes when acupuncture was performed around the time of oocyte retrieval. The other four meta-analyses, published by El-Toukhy et al. (2008) (13 trials, 2,500 participants), Cheong et al. (2010) (14 trials, 2,670 subjects), El-Toukhy and Khalaf (2009), and Sunkara et al. (2009) (14 trials, 2,870 subjects), also could not confirm a beneficial effect from using acupuncture during IVF. This meta-analysis by Zheng et al. (2012), however, provided further clarification about the positive effects of acupuncture on IVF outcome and confirmed the benefits of acupuncture on IVF outcomes.

In conclusion, the literature from the last decade investigating the effects of acupuncture on fertility and other physiology related to reproduction has been positive and has shown benefits of acupuncture used as a sole therapy or in conjunction with ART. Acupuncture has also been established to be safe without any adverse effects (Lim and Wong, 2010).

Chinese Herbal Medicine and Fertility

Chinese herbal medicine (CHM) is one of the main therapeutic modalities used in Chinese medicine along with acupuncture. In recent years, CHM has become one of CAM modalities that infertile couples commonly seek and use in the West (Smith et al., 2010). Consequently, increasing numbers of scientific research have been engaged on various Chinese herbs and formulas to find out their biochemical functions and therapeutic effects. However, very little evidence based research exists in the West that demonstrates the effectiveness and pharmacology of CHM in treating human female infertility (Wing, 2009).

One of the infertility patterns associated with AMA is the declining of Tian Kui and the Kidney Essence. As an overall pattern, Kidney deficiency has been found to be the most common diagnosis among infertility (Maciocia, 20110). The herbs commonly used to treat infertility associated with Kidney deficiency belong to herbal categories of tonifying herbs including Dang Gui (*Angelicae Sinensis Radix*), Shu Di Huang (*Rehmanniae Radix preparata*), Gou Qi Zi (*Lycii Fructus*), Wu Wei Zi (*Schisandrae Fructus*), Shan Yao (*Dioscoreae Rhizoma*), and Shan Zhu Yu (*Corni Fructus*). These herbs are often combined and used in herbal formulas for treating infertility. Although the exact mechanisms and effectiveness of these herbs for treating infertility have not been clearly established by research, there are possible mechanisms that can explain the benefits of these herbs for fertility. The potential mechanisms involved in the pharmacology of Chinese herbs for supporting fertility include antioxidant activity and benefits on the endometrial environment.

Chinese Herbs and Antioxidant Activity

Many of the herbs used for treating infertility in TCM have been found to exhibit antioxidant activity, which is one of the potential benefits to fertility. Antioxidants can reverse the damaging effects of oxidative stress (OS). OS has been associated with decreased fertility in both male and female (Ruder, Hartman, & Goldman, 2009). OS also influences oocyte and embryo quality and thus the fertilization rates (Agarwal, Gupta, & Sharma, 2005), and antioxidant protection is required at sites of gametogenesis, fertilization, and implantation (Taylor, 2001). Antioxidant capacity of follicular fluid during oocyte retrieval has also been found to be associated with characteristics of in-vitro fertilization (IVF) success (Ruder, Hartman, & Goldman, 2009). OS is caused by generation of reactive oxygen species (ROS), which are naturally generated at both endogenous and exogenous levels (Venkatesh *et al.*, 2009). In fact,

ROS serve as both physiological and pathological roles in female reproductive tract; ovaries, fallopian tubes, and embryos, and pregnancy (Agarwal, Gupta, & Sharma, 2005). However, when the balance between ROS and antioxidants is disrupted, overabundance of ROS leads to OS, which is damaging to DNA, proteins, cell membranes (Ebisch, 2007) and to reproductive functions. Antioxidants, their cofactors, and certain enzymes are capable of disposing, scavenging, or suppressing the formation of ROS (Ruder, Hartman, & Goldman, 2009; Lavente, 2012) and provide protection of cells against oxidative stress (Ebisch, 2007), and antioxidants can be beneficial for normalizing and enhancing reproductive functions. Many Chinese herbs used for enhancing fertility and treating infertility related conditions, such as Dang Gui (*Angelicae Sinensis Radix*), Shu Di Huang (*Rehmanniae Radix preparata*), Wu Wei Zi (*Schisandrae Fructus*), and Gou Qi Zi (*Lycii Fructus*) have shown to have strong antioxidant activity (Kobayashi et al., 1993; Hou et al., 2004; Mathew & Abraham, 2004; Luo et al., 2006). This is one possible scientific foundation explaining the therapeutic effects and benefits of Chinese herbs for fertility.

Dang Gui (*Angelicae Sinensis Radix*) contains ferulic acid as one of the major chemical constituents, which exhibits strong antioxidant activity as well as neuroprotective functions (Kobayashi et al., 1993; Hou et al., 2004; Mathew & Abraham, 2004). Shu Di Huang (*Rehmanniae Radix preparata*) also contains many antioxidant enzymes such as ascorbate peroxidase (APX), glutathione reductase (GR), non-specific peroxidase (POX), and superoxide dismutase (SOD) (Choi et al., 2004). These antioxidant enzymes neutralize excessive ROS and prevent it from damaging the cellular structure (Agarwal, Gupta, & Sharma, 2005). The pharmacological effects of Wu Wei Zi (*Schisandrae Fructus*) can be attributed to its lignan constituents, particularly the dibenzocyclooctadiene-type lignans (Lu & Chen, 2009). The

dibenzocyclooctadiene-type lignans show antioxidative functions as well as hepatoprotective functions (Lu & Chen, 2009). Gou Qi Zi (*Lycii Fructus*) has also been shown to have strong antioxidant property from its potential bioactive components, such as arabinogalactanproteins (AGPs), carotenoid zeaxanthin, and vitamin C precursor 2-O-(beta-D-glucopyranosyl) ascorbic acid (Bucheli et al., 2011). The water decoction of Gou Qi Zi (*Lycii Fructus*) has also been found to significantly increase the activity of glutathione peroxidase in animals' blood serum, decrease the lipid peroxide content, and increase the activity of superoxide dismutase in the blood (Bucheli et al., 2011). This potent antioxidant property of Gou Qi Zi (*Lycii Fructus*) explains its potential benefits to support reproductive functions. Furthermore, chemical constituents of Shan Yao (*Dioscoreae Rhizoma*) include saponins, such as aglycon and diosgenin, phenolic compounds, sterols, and polysaccharides (Bensky, Clavey, & Stoger, 2004), and its antioxidant activity and DNA protective effects has been demonstrated by Szeto et al. (2009).

Shan Zhu Yu (*Corni Fructus*) is another herb commonly used for infertility treatment for Kidney deficiency pattern in Chinese Medicine. Major chemical constituents of this herb include volatile constituents, such as isobutanol and isoamyl alcohol, glycosides and aglycons, tannins, organic acids, vitamin A, polysaccharides and more (Bensky, Clavey, & Stoger, 2004). Peng, Wei, & Lau (1998) found antioxidant activities of Shan Zhu Yu (*Corni Fructus*) against oxidative stress in macrophages and endothelial cells, which demonstrates its potential benefit for fertility.

Chinese Herbs and their Benefits on Blood Flow

Chinese herbs, such as Dang Gui (*Angelicae Sinensis Radix*), have also been found to improve blood flow to the uterus and have anti-spasmodic effect on the uterus in rat (Du *et al.* 2006). For instance, Dang Gui (*Angelicae Sinensis Radix*) has been found to improve

microcirculation via opening capillaries (Shi, Zheng, Cai, & Wu, 1995). Ferulic acid found in Dang Gui (*Angelicae Sinensis Radix*) has been found to significantly improve blood fluidity, inhibit platelet aggregation, decrease serum lipids, and prevent thrombus formation (Kobayashi et al., 1993; Hou et al. 2004; Mathew & Abraham, 2004). Z-ligustilide is another chemical constituent of Dang Gui (*Angelicae Sinensis Radix*) (Wu & Hsieh, 2011), and it inhibits spontaneous contraction of the uterus (Du et al. 2006). Z-ligustilide may also inhibit prostaglandin F-2 , oxytocin, acetylcholine chloride and potassium depolarization-induced uterine contraction, suggesting that ligustilide modulates the function of uterine tissue and has a non-specific anti-spasmodic effect (Shi et al., 1995). Furthermore, Z-ligustilide has also been found to improve microcirculation via opening capillaries (Shi et al., 1995). These scientific bases of Dang Gui (*Angelicae Sinensis Radix*) correlate with TCM function of invigorating and harmonizing blood. The possible anti-spasmodic effect on the uterus may correspond to the TCM function of regulating menses. Furthermore, Wu Wei Zi (*Schisandrae Fructus*) has also been found to inhibit platelet aggregation (Lu & Chen, 2009).

Chuan Xiong (*Chuan Xiong Rhizoma*) is another herb that invigorates blood and promotes the movement of qi (Bensky, Clavey, & Stoger, 2004) that is used to benefit fertility in CHM. The bioactive chemical constituents of Chuan Xiong (*Chuan Xiong Rhizoma*) include ligustilide and butylidenephthalide, which inhibit vasoconstrictions in vitro on rat abdominal aorta segments (Liang, He, & Yang, 2005). Ligustilide and butylidenephthalide significantly inhibit the vasoconstrictions induced by norepinephrine bitartrate (NE) and calcium chloride (CaCl₂) and relax the vasoconstrictions (Liang et al., 2005). This biochemical action explains the function of the herb to invigorate and regulate blood in TCM. Ran et al. (2011) also

indicates that Chuan Xiong (*Chuan Xiong Rhizoma*) also has antioxidant activity, which can be beneficial for supporting reproductive functions.

Although the exact mechanisms and effectiveness of CHM for infertility have not been established in the West, currently available literatures seem to support the benefits of CHM on various aspect of reproductive functions including the antioxidant activity and benefits to the blood flow. Furthermore, several research have been engaged for the last decade to assess the effects of CHM on fertility and pregnancy outcome and found that CHM is beneficial for normalizing reproductive functions and improving pregnancy outcome (Lian, 1991; Wing & Sedlmeier, 2006; Wing, 2009; Reid & Stuart, 2011; Chen, 2011).

Lian (1991)

An earlier study by Lian (1991) demonstrated the beneficial effects of CHM on the treatment of luteal phase defect and improving pregnancy outcome. In this cohort study, 32 women with simple luteal phase defect were treated with CHM for 3-6 months. The age of women ranged from 25 to 37 years, averaging 31 years, and the duration of infertility ranged from 1.5 to 8 years, averaging 3.5 years. All women in this study presented some forms of Kidney deficiency, and they were treated with CHM according to their differential diagnosis. After CHM treatment, statistically significant improvements were seen in their basal body temperature patterns such as in their hyper-thermal phase and transitional phase. Eighteen out of 32 women became pregnant, yielding to 56% pregnancy rate. Although there were weaknesses of this study such as the small sample size and lack of control, this study demonstrated the positive effects of CHM on female reproductive functions and pregnancy outcome.

Wing and Sedlmeier (2006)

Wing & Sedlmeier (2006) were the pioneers of research in the West to investigate the effects of CHM on fertility using scientific analyses. This study was a prospective cohort clinical study designed to measure bio-medical markers associated with female fertility and to determine if CHM can improve these physiological markers as well as yielding to pregnancy. This study involved 50 women with diagnosis of unexplained infertility. There were 18 patients who were under the age of 35 and 32 patients were older than 35. The data measured twelve fertility indicators including pre-ovulatory endometrial thickness (day 1), serum FSH, antral follicle counts (right ovary, left ovary, and total follicle count), the size of dominant follicle, post-ovulatory endometrial thickness (day 21), post-ovulatory uterine artery peak systolic velocity (PS), post-ovulatory uterine artery pulsatility index (PI), post-ovulatory serum progesterone, corpus luteum size and corpus luteum vascularity. The differences among these fertility indicators between pre- and post- treatments were compared and statistically analyzed.

The results of this study indicated significant positive differences between pre- and post-treatment measurements after 3 menstrual cycles. Significant reduction in serum FSH ($p < 0.001$) post-treatment indicated improved ovarian function. Significant increases in endometrial thickness ($p < 0.002$) as well as in uterine artery blood flow ($p < 0.001$) were also reported, indicating improved endometrium receptivity. Similarly, the blood flow resistance in the uterine artery or pulsatility index (PI) decreased significantly ($p < 0.001$), indicating improved quality of the endometrium via increased amount of estradiol supplied to the uterus. Progesterone is also important hormone that maintains the endometrium for implantation and pregnancy. In this study, progesterone level also increased significantly after treatments ($p < 0.001$). This increase in progesterone relates to increased corpus luteum size and increased

vascularity. In this study, corpus luteum size and vascularity also increased significantly post-treatment ($p < 0.001$). The total number of follicles developed by both ovaries in this study increased only marginally ($p = 0.05$); however, the size of the Graafian follicle increased significantly ($p < 0.001$). These positive changes were seen after three menstrual cycles. Furthermore, the study yielded 56% pregnancy rate after 6 months while there were no side effects reported from the herbal medicine administered in this study. Although the sample size of this study was relatively small and the herbal medicine used as treatments were unreported this study established an excellent foundation for future research on the effect of CHM on infertility.

Wing (2009)

Wing (2009) further investigated the effects of Chinese herbal medicine (CHM) on human endometrial receptivity to embryo implantation. This study was a prospective interventional clinical study. The participants of this study included total of 100 patients, 50 who had at least 2 failed IVF cycles and with diagnosis of unexplained infertility as a treatment group and 50 healthy women as a control group. The participants were grouped into three. The treatment group ($n=50$) received CHM while 50% of the reference group ($n=25$) received placebo treatment and 50% of the reference group ($n=25$) received CHM identical to the treatment group. Only the reference group was double blinded and controlled in this study. CHM treatments were given over three menstrual cycles. The median age for the treatment group in this study was age 42. The physiological parameters associated with reproductive functions were measured at pre- and post- treatment times. The measurements included serum FSH, serum progesterone, endometrial thickness (ET), sub-endometrial 3D-PDA ultrasound indices; vascularization index (VI), flow index (FI), vascularization flow index (VFI), and pregnancy

outcome. These measurements from pre- and post- treatment were compared and analyzed. The results of this study indicated statistically significant positive improvement between pre- and post- treatment measurements of the all physiological parameters measured ($p < 0.001$). There were no statistically significant changes or improvements seen among the placebo group and their pre- and post- treatment measurements. This study yielded 34% pregnancy rate among patients with failed IVF patients from CHM treatments over three menstrual cycles. Wing (2009) concluded that administration of CHM has a positive effect on the endometrial receptivity measures used in this study. No side effects from CHM administered were reported. This study further established the beneficial effects of CHM and demonstrated CHM as a beneficial and effective treatment intervention for patients with history of failed IVF cycles.

Later animal studies by Yu, Yang, and Yin (2011) also confirmed the positive effects of CHM on improving endometrial receptivity and embryonic implantation and thereby increasing pregnancy rate. In this animal study, CHM was shown to reverse the negative expression of the endometrium of mice with embryo implantation dysfunction.

Reid & Stuart (2011)

Earlier findings on the positive effects of CHM on pregnancy outcome by Wing and Sedlmeier (2006) and Wing (2009) were confirmed by the most recent systematic review by Reid & Stuart (2011). This systematic review was conducted to investigate the effects of CHM for the management of infertility. This study included total of 24 articles and 6 books including 8 randomized controlled trials (RCTs) involving 1005 participants, 13 cohort studies involving 793 participants, 3 case series, and 6 case studies that investigated treatments of infertility with TCM therapy. This review included studies that used CHM alone or, in combination with acupuncture or Western medicine (WM) in the form of drugs or surgery. The control group in RCTs and CTs

received western medicine pharmacological treatment only. The women in this review were between 18 and 45 years old, with a mean age of 30 years and had primary or secondary infertility. Associated conditions in this study included endometriosis, polycystic ovarian syndrome, amenorrhea, oligomenorrhea, luteal phase defect, fallopian tube blockage, pelvic inflammation, hyperprolactinemia, advanced maternal age, or unexplained infertility. Treatment duration with CHM was on average 4 months in the RCTs and 5-6 months in the case studies. Pregnancy outcome was measured, and the results revealed that the odds of achieving a pregnancy with CHM therapy over a 4-months period were 3.5 times higher than with Western medicine (WM) drug therapy in this review. The pregnancy rates in the CHM group were 60% compared with 32% in the WM group. This study demonstrated the overall benefits and CHM as an effective therapy for treating infertility.

TCM and ART

The positive effects of acupuncture on ART outcomes have been demonstrated by several studies as mentioned in the earlier part of this literature review. Combining CHM and acupuncture as an adjunct to ART for improved outcome is still new, and there are only a few literatures on this subject. However, the literature available so far have shown positive effects of adding both acupuncture and CHM to improve the outcome of ART.

Rubin (2010)

This study was a case study of 41-year-old woman with secondary infertility due to AMA and diminished ovarian reserve undergoing IVF. This study was one of the very few studies that demonstrated the safety and efficacy of combining CHM and acupuncture during IVF. Rubin (2010) combined individualized CHM therapy as well as modified acupuncture protocol from Paulus et al. (2002). The end result of this study was a delivery of a healthy baby girl. This

study showed the potential of using both CHM and acupuncture to improve and support pregnancy and live birth outcome of IVF cycle as well as AMA women with diminished ovarian reserve whose prognosis for pregnancy outcome of IVF is poor. The weakness of this study was small sample size since it was a case study. However, this study established a good foundation for future study for combining acupuncture and CHM to improve the outcome of ART.

Chen (2011)

Chen (2011) engaged in a retrospective chart review of 34 patients and compared the pregnancy outcomes when the combination of CHM and acupuncture are used with IVF versus acupuncture alone with IVF. The results showed a significantly higher pregnancy rate in the group that was treated with both acupuncture and CHM compared to the acupuncture only group. This study demonstrated the benefits of combining CHM and acupuncture during IVF cycles and confirmed the positive finding from the earlier case study by Rubin (2010).

TCM and Infertility related to Advanced Maternal Age (AMA)

Although AMA women were often included in the literatures reviewed above, to my best knowledge, there are only limited amount of articles available on treatment of AMA related infertility. Hesse (2006) demonstrated the effectiveness of CHM for achieving pregnancy with a single case study of 45-year-old woman with a history of poor ovarian response and unsuccessful IVF. The 45-year-old woman achieved pregnancy after taking CHM for 5 months. Another study that investigates the effect of TCM for advanced maternal age woman was carried out by Daghighi (2011). This was also a case study of 39-year-old woman with infertility. The study demonstrated that acupuncture and CHM therapy were effective in normalizing elevated FSH, which is a common hormonal status that AMA women present indicating low ovarian reserve. Although this study does not include a pregnancy outcome, the result demonstrating TCM's

ability to normalize AMA woman's hormonal status was a contribution to the existing literature. Earlier study mentioned above by Rubin (2010) was another case study example of how TCM can support and improve the pregnancy outcome of AMA women with diminished ovarian reserve undergoing IVF.

Literature Review Integration

Currently available studies on TCM indicate benefits and effectiveness of both acupuncture and CHM for improving reproductive functions and pregnancy outcome. Safety of acupuncture and CHM as a treatment modality for infertility has also been demonstrated. The mechanisms of actions seem to involve neuroendocrine system such as HPO axis, regulation of hormonal status and uterine and ovarian blood flow and receptivity, and improvement of follicular environment via antioxidant activity. Acupuncture and CHM therapy have been demonstrated to be effective treatment modalities for treating infertility or improving physiology associated with reproduction as a sole therapy or as an adjunctive therapy for ART. However, the literatures are often based on small sample size and lack randomization and control. The methodologies of some of the studies were also unclear, which affected the validity of the studies negatively. Therefore, there is a need for conducting studies that are well designed, randomized and controlled to improve the validity of the study to validate the current findings. A larger sample size also needs to be implemented for future studies to improve the validity and generalizability of the study. There are only limited articles available that investigate the effects of TCM therapy on infertility associated with advanced maternal age. Similarly, to my best knowledge, currently there are no articles available that specifically investigate the effective durations of TCM therapy on infertility related to advanced maternal age.

CHAPTER THREE: METHODOLOGY

Research Design

The purpose of the current research project is to compare the effects of different TCM therapy durations on pregnancy outcomes of infertile AMA women. The treatment durations compared were less than three months versus more than three months.

The current study was a retrospective chart review that engaged mixed methods of qualitative and quantitative research methods. The data source for the chart review was medical records from an established TCM clinic, Tao of Wellness, specializing in fertility treatment in Santa Monica, California, United States. This chapter further elaborates on the research design, sampling method, variables, population and sample, instrumentation as well as data collection and procedures. The qualitative research method was engaged in the analysis of data for developing qualitative interpretation. The quantitative method was engaged in the analysis of numerical data for causal explanation, prediction, and generalizability of data studied.

Rationale for the Use of Retrospective Chart Review

Retrospective chart review research design is an ideal method for gathering valuable data from archived clinic charts. This research design has many advantages that were ideal and appropriate for this study. The advantages include the following (Hess, 2004):

- Feasibility – Inexpensive since this method uses existing records.
- It is a suitable method for analyzing multiple outcomes.
- With retrospective chart review, it is easier to assess conditions where there is a long latency between the cause and the effect.
- This method helps the researchers generate hypothesis that can be then tested prospectively and initiates quality improvement for future study.

The current study engaged mix methods of qualitative and quantitative analysis leading to multiple outcomes, thus this study design was appropriate. Furthermore, this study involved a long latency between the cause and effect such as treatments and pregnancy outcome, so this study design was appropriate. Since this study was a pilot study, this research method allowed hypothesis to be generated and quality improvement for future study was assessed.

Conversely, there are disadvantages of a retrospective chart review as a research design that were important to acknowledge. The disadvantages acknowledge were as follows:

- With this study design, the investigator has to rely on accuracy of written record or recall of individuals (recall bias).
- The investigator has no control over exposure or outcome assessment.
- Important data may not be available or missing due to using data that was recorded not for the purpose of research.
- It is difficult to control bias and confounders due to lack of randomization and blinding.
- It is difficult to establish cause and effect.
- The study is observational in nature thus does not provide the same level of evidence as randomized controlled trials.

Despite the disadvantages of retrospective chart review as a study design, the advantage and the contribution of this pilot study outweighed the disadvantages.

Variables

The main independent variable engaged in this study was TCM treatment duration (less than 3 months or more than 3 months). The main dependent variable engaged in this study was pregnancy outcome (yes or no) determined by beta-hCG test or ultrasound. Other independent variables collected are as follows:

- Age
- Length of time spent trying to conceive before starting TCM therapy
- Previous reproductive history, such as pregnancy, miscarriage, and/or abortion
- Previous history of ART, IUI, controlled ovarian hyper-stimulation procedures, or ovulation induction medication usage
- Concurrent use of ART, IUI, controlled ovarian hyper-stimulation procedures, or ovulation induction medication along with TCM therapy
- TCM diagnosis assessed and recorded by the TCM practitioners at the clinic
- Western medical diagnosis given by western medical professionals (such as gynecologist or reproductive endocrinologists) and reported by the patients
- Acupuncture points prescribed on the first visit
- Chinese herbal medicine prescribed on the first visit
- Adverse effects reported by patients
- Positive remarks reported by patients
- Live birth outcome (yes, no, or unknown)

Sampling and Population

The medical charts of infertile AMA women who were treated at the TCM clinic between January 2008 and March 2012 were engaged for the study. The IRB proposal of this research was approved by the Yo San University IRB board (See attachment 1). Furthermore, the permission for the use of medical charts for this research was also obtained from the Tao of Wellness clinic (See attachment 2) before the data collection began.

Convenience, purposive sampling (non-probability sampling method) was used for this study. With a convenience sampling, it is difficult to ensure an accurate representation of a

larger population compared to random sampling (Black, 1999); however, convenience sampling is an appropriate method chosen for my study because of its feasibility. Convenient sampling has an advantage of being an inexpensive method that can ensure sufficient numbers of a sample for a study (Black, 1999) and a quicker sampling method than random sampling to collect sample population needed for a study. Furthermore, convenience sampling method is appropriate especially for a pilot study (www.experiment-resources.com). Convenience sampling allows investigation of phenomenon or qualities that occur within a given sample that can be used to detect relationships among different variables, and the findings can further be used for designing a future prospective or randomized study (www.experiment-resources.com). Purposive sampling (non-probability sampling) was used to select charts according to the inclusion and exclusion criteria of the study. Purposive sampling is an appropriate method for the current study to ensure the collection of specific characteristics and data needed to answer my research question for the study.

Sample size needed to be sufficiently large enough to make the outcome statistically significant yet achievable within the time frame allocated to complete the study. My goal was to review at least 200 charts to collect at least 30 charts for each of the main independent variables (1 to less than 3 months treatment group and more than 3 months treatment group).

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria were established in order to ensure the collection of data that were pertinent to the study.

1) Inclusion criteria

Patients who met the following criteria were included for this study.

- Infertile AMA females. Infertility among AMA females refers to at least six months of

unprotected intercourse without conception.

- Patients who had the diagnoses of secondary conditions related to infertility such as endometriosis, fibroids, tubal blockage, poor ovarian reserve (High FSH, Low AFC, Low AMH) and PCOS all were included in this study.
- All types of TCM diagnoses
- Patients who have received the following TCM therapy protocol at the clinic for at least 1 month;
 - Weekly acupuncture treatment of 30-45 minute at least 3 times per month.
 - Regular intake of customized Chinese herbal medicine formula. This factor was determined by the refilling record of patients.
- Patients who used ART, which includes IVF, GIFT, ZIFT, FET, and ICSI more than three months prior to beginning TCM therapy. Three-month gap was included as criteria in order to rule out the effects from the medications used during ART procedures.
- Patients who used IUI with controlled ovarian hyper-stimulation medications or without such medications or patients who used ovulation induction or hyper-stimulation medications alone more than three months prior to beginning TCM therapy. Three-month gap was included as criteria in order to rule out the effects from the medications used during ovarian hyper-stimulation medications used for IUI procedures.
- Patients who used ART, IUI, ovarian hyper-stimulating medications, and/or ovulation induction medication while receiving TCM therapy.

2) Exclusion criteria

The following charts of patients were excluded in this study;

- Patients who used TCM therapy less than 1 month

- Patients who used TCM therapy of acupuncture and CHM irregularly
 - Weekly acupuncture treatment less than 3 times per month
 - Irregular intake of herbal medicine determined by lack of regular refills on their records
- Patients who have received only acupuncture treatment as TCM therapy for fertility.
- Patients who have received only Chinese herbal therapy as TCM therapy for fertility.
- Patients who have received other modalities of TCM fertility assisting therapy such as Chi Gong and Fertility massage on a regular basis (>2 times a month)
- Patients who used egg donor or surrogacy during TCM therapy
- Patients who have known male factor infertility such as low sperm count, low morphology, and/or low motility confirmed by semen analysis.
- Patients whose male factor is unknown
- Patients who used ART procedures less than three months prior to beginning TCM therapy.
- Patients who used IUI with ovarian hyper-stimulating medications within less than three months prior to beginning TCM therapy.
- Patients who used ovarian hyper-stimulation medications or ovulation induction medication such as Clomiphene citrate within less than three months prior to beginning TCM therapy.

TCM therapy used in this study

TCM therapy used in this study included acupuncture and CHM that is customized to each patient according to patients' TCM diagnoses. The treatments were given by 10 TCM licensed practitioners whose clinical experiences varied from 2 years to more than 20 years.

Disposable stainless steel acupuncture needles of 0.18mm x 25mm were used for acupuncture treatments. Each acupuncture session lasted 30-45 minutes. The manipulation techniques used were unknown and unrecorded on the medical charts. Chinese herbal medicine formulas consisted of 10-15 herbs which were formulated according to patients' diagnosis. The herbal formulas were given either in dried whole form, granulated powdered form, or in capsule form. Most common form of herbal formula given was in dried whole form and secondly the granulated powdered form. Patients were given an instruction on cook the dried whole form of herbal formula to make herbal decoction at home. Written instruction on how to cook and take herbal medicine was given to each patient.

Instrumentation

A chart abstraction log and a data collection log were used as instruments for this study (See Appendix 1 and 2). A chart abstraction log was used to collect pertinent information of both independent and dependent variables from the charts. The chart abstraction log was designed so that the investigator could clearly, precisely, and systemically collect and record specific data needed from the charts. To ensure validity and reliability of the data used for this study, it was crucial to develop and utilize instruments that could collect pertinent information. The use of chart abstraction log as an instrument ensured systematic and precise data collection process from the medical charts that were recorded not for research purposes. The main outcome measurement, pregnancy outcome (yes or no), was recorded on the chart abstraction log. Treatment durations in days until the confirmation of pregnancy or last menstrual period before discontinuation of TCM therapy were recorded on the data abstraction log. Other variables listed earlier in this chapter were also recorded to give more insights about this study.

After data were collected and recorded on the chart abstraction logs, data were further organized using the data collection log (See appendix 2). Patients were grouped according to treatment types; either a group of patients who had some forms of Western medical fertility assisting treatments (ART, IUI, and/or controlled ovarian stimulation (COS)) during the course of TCM treatments (Group A) or patients who had only TCM therapy (Group B) (See table 1 below). Patients were also organized according to different treatment durations they went through (Group a, b, or c). Patients who had TCM therapy for 1 month to less than 3 months were grouped as group-a, 3-6 months treatment duration group was group-b, or 7 months or more treatment duration group as group-c. These three groups with different TCM treatment durations were applied for an organization purpose (See table 1 below). The main dependent variable, the pregnancy outcome (yes=1, no=2), was recorded on the data collection log as well as other independent and dependent variables listed earlier in this chapter. An identification number was assigned depending on the group to which the patient belonged. This was done in order to help organize and de-identify data. This data collection log was used to organize information for quantitative analysis to assess statistical significance among the independent variables for the outcome measured. The data collection log was also designed to ensure precise record and organization of the data needed for statistical analysis.

Table 1. Data Collection Log Organization Criteria

TX type	ID code for a patient	TX duration	Other variables
TCM therapy + ART, IUI, and/or COS	A-a + number code given for a patient	1- < 3 months	<u>Main dependent variable:</u> Pregnancy outcome
	A-b + number code given for a patient	3-6 months	
	A-c + number code given for a patient	7+ months	
TCM therapy only	B-a + number code given for a patient	1- < 3 months	<u>Other independent variables:</u> age, reproductive history etc.
	B-b + number code given for a patient	3-6 months	
	B-c + number code given for a patient	7 + months	

A master list and de-identified number code list were kept in separate places in order to preserve the confidentiality of the patients’ name and medical record. The master list and de-identified number code list were kept at separate three offices where data can be secured in locked filing cabinets with limited access. These three offices were the clinic manager’s office, capstone advisor’s office, and the investigator’s office. All the records stored in the computer were backed up every time data were collection took place. An USB device was used for a backup in case of unexpected computer failures.

Data Collection Procedures

Data collection began in June 1st of 2012 and was completed by November 1st of 2012 at the Tao of Wellness Clinic, Santa Monica, CA. The timeline and activities that took place is as follows (see Table 2 below).

Table 2. Timeline and Data Collection Procedures

Timeline	Data collection activity
<p>June 1st 2012 – June 30th 2012</p>	<p>All of the fertility patients' charts who were treated between January 2008 and March 2012 were reviewed for the eligibility for the study according to the inclusion criteria.</p>
<p>July 1st 2012– September 31st 2012</p>	<p>Charts who met the inclusion criteria were selected for further review and data abstraction using the data abstraction log (See Appendix 1).</p>
<p>October 1st 2012– October 30th 2012</p>	<p>Data were further recorded and organized using the data collection log (Appendix 2). IC code was given to each patient to de-identify names of patients. A master list and IC code list were created.</p>
<p>November 1st 2012– November 30th 2012</p>	<p>Data analysis using data collection log and statistical analysis</p>
<p>December 1st 2012- December 31st 2012</p>	<p>Results were reviewed and organized</p>

CHAPTER FOUR: DATA ANALYSIS AND RESULTS

Data overview

The main objective of this study was to determine the effects of TCM treatment durations on pregnancy outcomes of infertile AMA women. The hypothesis engaged for this study was that treatment durations of more than 3 months would yield to more pregnancy outcomes than less than 3 months treatment duration.

This study engaged in a retrospective chart review using convenient, purposive sampling. A total of more than 500 patients' charts were reviewed for this study. Charts that fit the inclusion criteria of this study were chosen as eligible for further data abstraction and collection process. Out of the 500 charts, 67 charts were qualified to fit the inclusion criteria for this study. Pertinent data were gathered from each chart using the chart abstraction log (see appendix 1). Once the data were gathered on the chart abstraction logs, they were further organized into groups and subgroups using data collection log (see appendix 2). Dependent variables and other independent variables were recorded on the data collection log. Finally, both descriptive and inferential statistical methods were engaged to analyze the data.

Statistical Analyses

Both descriptive statistical analysis and inferential statistical analysis, such as univariate and bivariate analyses, were used to analyze both qualitative and quantitative data. Firstly, descriptive statistical analyses were engaged in order to present the demographics and characteristics of the patient population in this study. The univariate statistical technique using percentage, mean, median, minimum and maximum values were used to describe the patient population of this study. The quantitative data included continuous variables such as age and previous reproductive history including the number of prior pregnancy, miscarriage, abortion,

and live births. The number of previous and concurrent ART and other medical intervention used along with TCM therapy were also analyzed. The qualitative data analyzed in this section include categorical and bivariate data such as pregnancy outcome (yes or no), live birth outcome (yes or no), previous or current ART use (yes or no). Categorical data analyzed included such as types of western fertility treatment used (IVF, IUI, and/or COS). Descriptive analysis was engaged to present Western diagnosis, TCM diagnosis, acupuncture points used, and Chinese herbs used.

Secondly, inferential statistical analyses were engaged in order to report the main results of this study. These analyses determined the effects of different TCM treatment durations (less than three months vs. more than three months) on the pregnancy outcomes (yes or no) of infertile AMA women. In order to rule out the age effect on pregnancy outcome, the data are also analyzed and reported according to age groups within the treatment duration groups. Similarly, the effects of the concurrent use of ART or other medical fertility assisting procedures along with TCM therapy on pregnancy outcomes were ruled out. Furthermore, exploratory analyses were also engaged to determine the effects of TCM treatment durations on live birth outcomes.

Tables are included to present the results in this section as well as additional graphs and charts. Readers should note that the graphs and charts do not display exact figures but are merely a visual representation.

Descriptive Statistical Results

This section presents the main descriptive statistical analyses and the results of the study. The purpose of this section was to describe the characteristics of the women in this study. The univariate statistical technique using the percentage, mean value, median value, minimum value

and maximum value is used in this section. Median value is used to represent the central tendency of the data and when there is a skewness of data.

1. Age

Out of the 67 patients in this study, the median age was 40, while the youngest age of patient was 35 and the oldest was 47 (see Table 3 below).

Table 3. Age of Patients

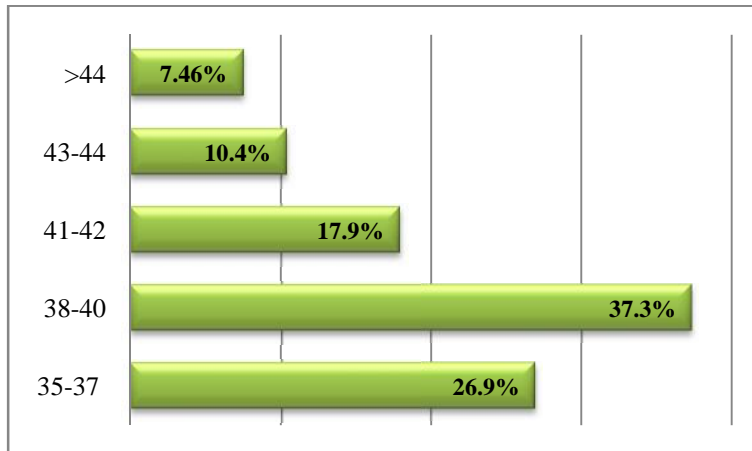
<i>Variable</i>	<i>Median</i>	<i>Minimum</i>	<i>Maximum</i>
Age	40	35	47

Patients’ age was further organized into groups of 35-37, 38-40, 41-42, 43-44, and 45-47 (see Table 4 below). The most common AMA group in this study was age 38-40 (37.3%) and the second common group was age 35-37 (26.9%). A bar graph below (Illustration 1) is used to present the general proportions of age groups in this study.

Table 4. Age Groups

	<i>N</i>	<i>Percent</i>
<i>Age groups</i>		
35-37	18	26.9 %
38-40	25	37.3%
41-42	12	17.9%
43-44	7	10.4%
44-47	5	7.46%
Total	67	100.0%

Illustration 1. Age Groups



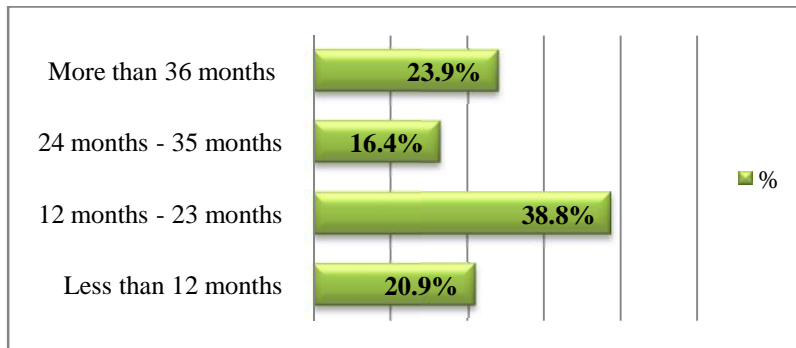
2. Reproductive History

Reproductive history of the patients in this study before seeking TCM treatments is reported below. The median number of months trying to conceive before seeking TCM therapy was 18 months. The most common range of months patients tried to conceive was 12-23 months (see Table 5 and Illustration 2 below).

Table 5. Months Patients Spent Trying to Conceive before Seeking TCM Treatment

Months	<i>N</i>	<i>Percent</i>
Less than 12 months	14	20.9%
12 months - 23 months	26	38.8%
24 months - 35 months	11	16.4%
More than 36 months	16	23.9%
Total	67	100.0%

Illustration 2. Months Patients Spent Trying to Conceive Prior to Seeking TCM Therapy

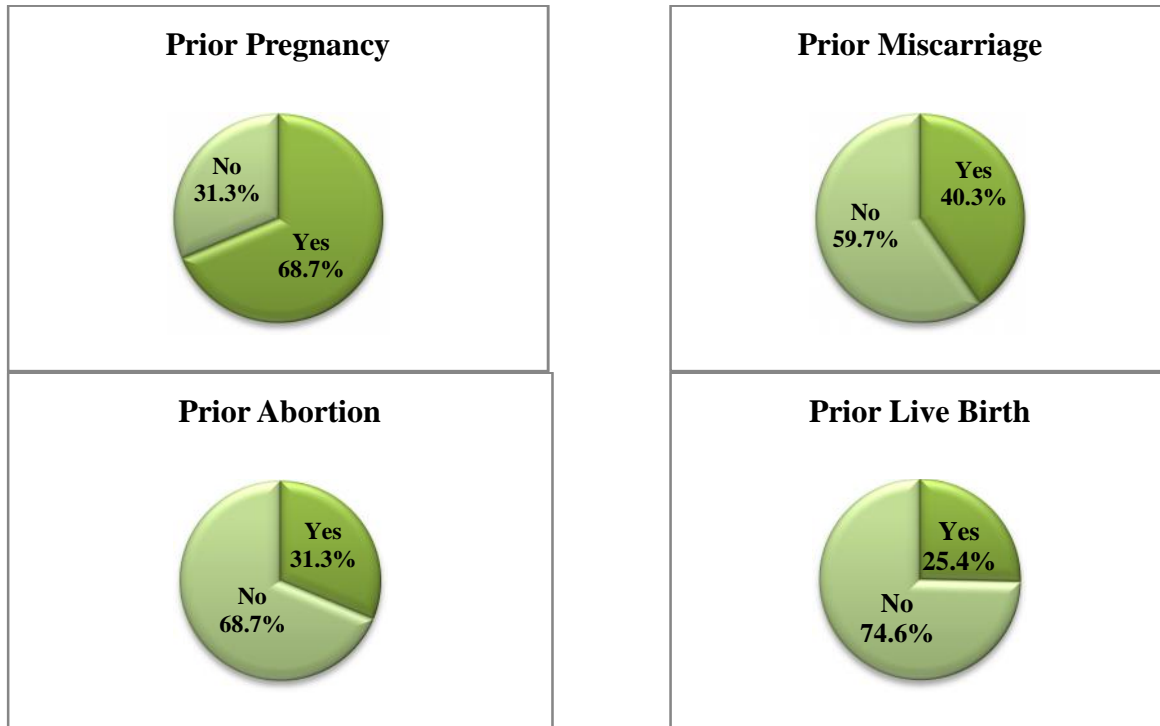


As shown in Table 6 and Illustration 3 below, 68.7% of women had prior pregnancy ($N=46$), 40.3% had miscarriage ($N=27$), 31.3% had abortion ($N=21$) and 25.4% had prior live birth ($N=17$) before seeking TCM treatments.

Table 6. Summary of Reproductive History

	<i>Yes</i>		<i>No</i>	
	<i>N</i>	<i>%</i>	<i>N</i>	<i>%</i>
History of prior pregnancy	46	68.7%	21	31.3%
History of miscarriage	27	40.3%	40	59.7%
History of abortion	21	31.3%	46	68.7%
History of live birth	17	25.4%	15	74.6%

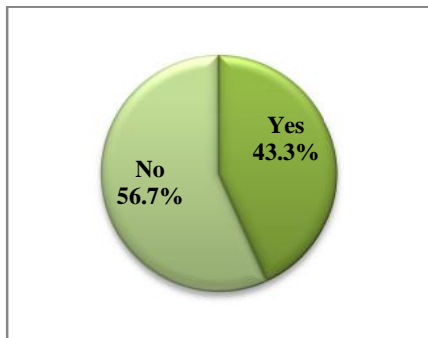
Illustration 3. Reproductive History



3. History of ART or other medical fertility treatments use before seeking TCM therapy

Out of 67 patients in this study, 43.3% of the patients ($N=29$) used some forms of ART, and/or IUI, and/or COS procedures before seeing TCM therapy while 56.7% of the patients ($N=38$) did not use any such medical procedures (see Illustration 4 below).

Illustration 4. Prior use of ART, IUI and/or COS before seeking TCM therapy



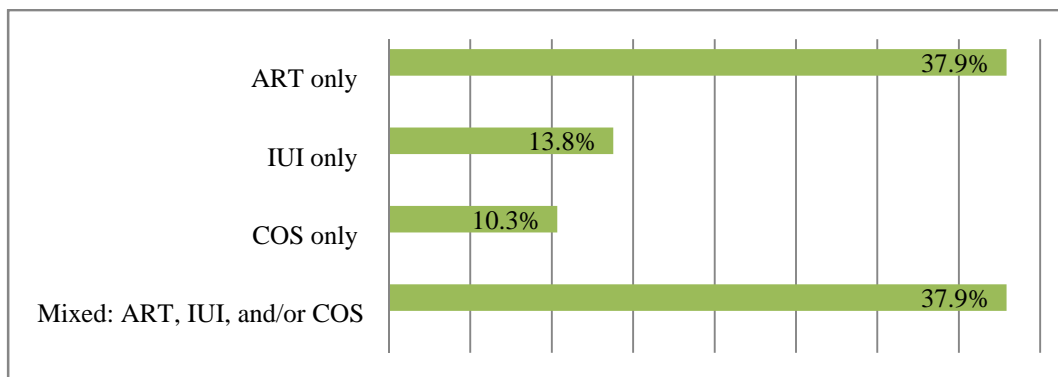
Types of prior ART use before seeking TCM therapy

Western medical procedures used by the patients in this study prior to seeking TCM therapy include IVF, IUI, and COS. Table 7 below reports the types of medical procedures used prior to seeking TCM therapy. Out of the patients who used Western fertility assisting procedures prior to TCM therapy, 37.9% of them used IVF only ($N=11$), 13.8% of the patients used IUI only ($N=4$), 10.3% used COS only ($N=3$), while 37.9% of the patient ($N=11$) used combinations of treatments over time such as IVF, IUI, and/or COS (see Table 7 and Illustration 5 below).

Table 7. Types of Western fertility treatments used prior to TCM therapy

	<i>N</i>	<i>Percent</i>
<i>Western fertility treatment types</i>	<i>ART only</i>	11 37.9%
	<i>IUI only</i>	4 13.8%
	<i>COS only</i>	3 10.3%
	<i>Mixed (ART, IUI, and /or COS)</i>	11 37.9%
<i>Total</i>	29	100.0%

Illustration 5. Types of Western fertility treatments used before seeking TCM therapy



Furthermore, the numbers of ART and fertility assisting procedures used before seeking TCM therapy is presented below (see Table 8 below). The median numbers of ART cycles used prior to TCM therapy was 2 cycles. For IUI treatments and COS treatments, the median numbers of cycles used were 3 cycles and 3 cycles respectively.

Table 8. Western fertility treatment types and number of cycles used prior to TCM therapy

		<i>Median</i>	<i>Minimum</i>	<i>Maximum</i>
<i>Treatment types and number of cycles used</i>	<i>ART</i>	2	1	6
	<i>IUI</i>	3	1	6
	<i>COS</i>	3	1	4
	<i>FET</i>	1.5	1	2

4. Western Medical Diagnoses

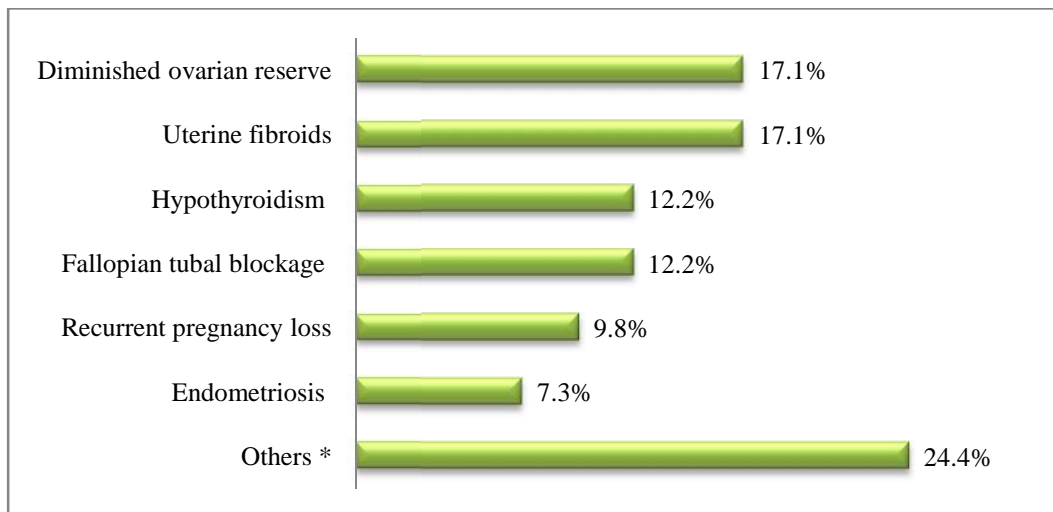
The most common western medical diagnoses of the patients reported in this study were diminished ovarian reserves and uterine fibroids. Hypothyroidism, fallopian tubal blockage, recurrent pregnancy loss, and endometriosis were also common (see Table 9 and Illustration 6 below). These diagnoses were given by either gynecologists or reproductive endocrinologists and were reported by the patients themselves on their first visits. This section of the current study only reports the available findings in order to describe the patient demographics in this study, therefore, further analyses of the Western medical diagnoses are not in the scope of this study.

Table 9. Western Medical Diagnoses Reported

	<i>N</i>	%	
Medical Diagnoses	Diminished ovarian reserve	7	17.1%
	Uterine fibroids	7	17.1%
	Hypothyroidism	5	12.2%
	Tubal blockage	5	12.2%
	Recurrent pregnancy loss	4	9.8%
	Endometriosis	3	7.3%
	Others*	10	24.4%
Total	41	100.0%	

* Others include PCOS, Luteal defect, Immunology factors such as elevated NK cells and ANA, Hashimoto thyroiditis, adenomyosis, hydrosalpinx, secondary amenorrhea, uterine polyp, thalassemia

Illustration 6. Western Medical Diagnoses Reported



* Others include PCOS, Luteal defect, Immunology factors such as elevated NK cells and ANA, Hashimoto thyroiditis, adenomyosis, hydrosalpinx, secondary amenorrhea, uterine polyp, thalassemia

5. TCM Diagnoses

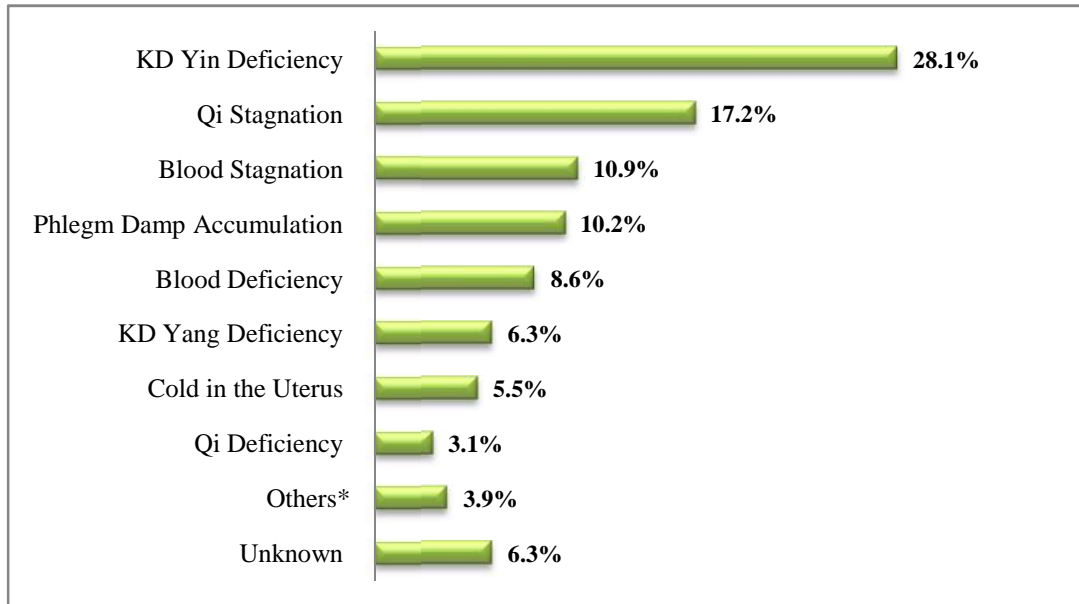
TCM diagnoses of the patients in this study are reported below (see Table 10 and Illustration 7 below). The most common diagnosis was Kidney Yin Deficiency (28.1%) followed by Qi Stagnation (17.2%) and Blood Stagnation (10.9%). Phlegm-Dampness Accumulation (10.2%) and Blood Deficiency (8.6%) were also commonly seen. In some patients, the diagnoses were not reported (unknown, 6.3%). This section of the current study only reports the available findings from the medical charts in order to describe the patient demographic in this study, therefore, further analyses of TCM diagnoses are not in the scope of this study.

Table 10. TCM Diagnoses

		<i>N</i>	%
TCM Diagnoses	KD Yin Deficiency	36	28.1%
	Qi Stagnation	22	17.2%
	Blood Stagnation	14	10.9%
	Phlegm Damp Accumulation	13	10.2%
	Blood Deficiency	11	8.6%
	KD Yang Deficiency	8	6.3%
	Cold in the Uterus	7	5.5%
	Qi Deficiency	4	3.1%
	Others*	5	3.9%
	Unknown	8	6.3%
Total		128	100.0%

*Others include Kidney Jing Deficiency, Deficiency Heat, and Stomach Fire

Illustration 7. TCM Diagnoses



*Others include Kidney Jing Deficiency, Deficiency Heat, and Stomach Fire

6. Acupuncture treatment

The most common acupuncture points used in this study included points along the Conception vessel such as Ren-4, Ren-6, Ren-3, and Ren-12, points along Stomach channel such as ST-36, ST-25, ST-27, ST-28, and ST-29, and points along Spleen channel such as SP-9 and SP-6. Extra points such as Zi Gong Xue and Yin Tang were also commonly used. Other points used also include Du-20 and LI-11. This section only reports the findings from the data that are pertinent to describe the general treatments used in this study. Therefore, further analyses of acupuncture treatments are not in the scope of this study.

7. Chinese Herbal Therapy

Chinese herbal pharmacopoeia include more than 400 herbs (Bensky et al., 2004). In this study, patients received customized herbal formulas according to their TCM diagnoses. The most common form of herbal medicine used was dried, whole herbal formulation style which

generally consisted of 8-12 herbs. These formulations were decocted and made into tea forms by patients themselves. Occasionally, herbal medicine in granulated form, in capsule, or in pill form was also used. More than 90 different types of herb usage were observed from patient's herbal prescription given on their first visit in this study. Types of herbs prescribed on patients' first visit were recorded. The 11 most commonly prescribed herbs in this study were Dang Gui (*Angelicae Sinensis Radix*), Bai Shao (*Paeoniae Radix alba*), Chen Pi (*Citri reticulatae Pericarpium*), Gan Cao (*Glycyrrhizae Radix*), Chuan Xiong (*Chuanxiong Rhizoma*), Shu Di Huang (*Rehmanniae Radix preparata*), Gou Qi Zi (*Lycii Fructus*), Wu Wei Zi (*Schisandrae Fructus*), Tu Si Zi (*Cuscutae Semen*), Shan Zhu Yu (*Corni Fructus*), and Shan Yao (*Discoreae Rhizoma*) (see Table 11 below).

Table 11. The 11 Most Commonly Used Chinese Herbs in This Study

<i>Herbs</i>	<i>f</i>
Dang Gui (<i>Angelicae Sinensis Radix</i>)	56
Bai Shao (<i>Paeoniae Radix alba</i>)	50
Chen Pi (<i>Citri reticulatae Pericarpium</i>)	45
Gan Cao (<i>Glycyrrhizae Radix</i>)	45
Chuan Xiong (<i>Chuanxiong Rhizoma</i>)	42
Shu Di Huang (<i>Rehmanniae Radix preparata</i>)	38
Gou Qi Zi (<i>Lycii Fructus</i>)	34
Wu Wei Zi (<i>Schisandrae Fructus</i>)	25
Tu Si Zi (<i>Cuscutae Semen</i>)	24
Shan Zhu Yu (<i>Corni Fructus</i>)	22
Shan Yao (<i>Discoreae Rhizoma</i>)	20

This section of this study reports the findings regarding herbal therapy in order to provide information on the general view of herbal treatments used among patients in this study. Therefore, further analyses of herbal treatments are not the scope of this study.

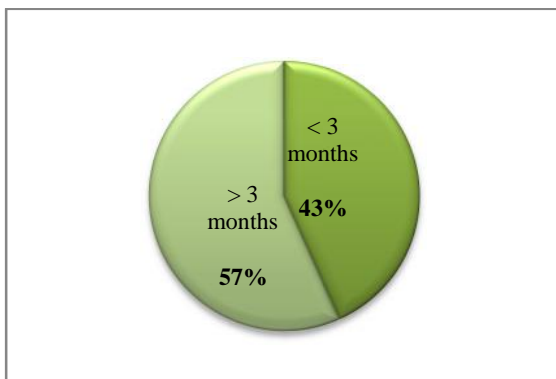
Inferential Statistical Results

Inferential statistical analyses were used to examine associations between treatment durations and pregnancy outcome in this study. Furthermore, probability value was analyzed to further determine the strength of the relationship between two variables and significance of the statistics.

1. Effects of Treatment Durations on Pregnancy Outcome

The main objective of this study was to determine whether or not different durations of TCM therapy lead to a significant change on pregnancy outcomes of infertile AMA women. In order to examine the associations between treatment durations and pregnancy outcomes the data were further organized into two groups with different lengths of TCM treatments; less than 3 months group and more than 3 months group. Out of the 67 patients in the study, 43% ($N=29$) of the patients had less than 3 months (1 month to less than 3 months) of TCM therapy while 57% ($N=38$) of the patients had more than 3 months of TCM therapy (see Illustration 8 below).

Illustration 8. Treatment duration groups in this study (< 3 Months vs. > 3 Months)



In order to determine whether or not TCM treatment duration of more than 3 months yielded to more pregnancies, a Pearson chi-square analysis was conducted between pregnancy outcome (yes vs. no) and treatment duration (less than 3 months vs. 3 months or more). Pearson chi-square analysis was chosen as appropriate analysis to compare categorical data such as pregnancy outcome and duration of treatment. As shown in Table 11 below, no significant difference in pregnancy outcome was found based on the duration of treatment ($\chi^2(1) = 0.88, p = .347$).

Table 11. Comparison of Pregnancy Outcomes by TCM Treatment Durations

(< 3 Months vs. > 3 Months)

			<i>Pregnant</i>		<i>Total</i>
			<i>Yes</i>	<i>No</i>	
Treatment Duration	< 3 months	Count	14	15	29
		%	48.3%	51.7%	100.0%
	3+ months	Count	14	24	38
		%	36.8%	63.2%	100.0%
Total		Count	28	39	67
		%	41.8%	58.2%	100.0%

Pearson Chi-Square (1) = 0.88, $p = .347$

2. Effects of TCM Treatment Durations on Pregnancy Outcome and Age Effect

In order to rule out an age effect that might be skewing the pregnancy results, a t-test on years of age was conducted between patients who became pregnant and those who did not. T-tests were chosen as appropriate analysis for comparing groups on continuous variables such as

age. As shown in Table 12 below, there was a significant age difference between the two groups ($t(65) = -2.19, p = .032$). The women who became pregnant were significantly younger (mean age = 38.7) compared to those who did not (mean age = 40.2).

Table 12. Age Comparison by Pregnancy Outcome

<i>Pregnant</i>	<i>N</i>	<i>Mean</i>	<i>SD</i>	<i>t</i>	<i>df</i>	<i>p-value</i>
Yes	28	38.68	2.5	-2.19	65	0.032
No	39	40.26	3.2	----	---	---

To control for the age effect and determine the effects of treatment durations on pregnancy outcomes, women were further separated into two groups using a median split for age. Women aged 35 to 39 formed one group, and women aged 40 to 47 formed the other group. Table 13 below reports the pregnancy outcome (yes or no) by the treatment durations within each age group. Consequently, two chi square analyses were conducted between pregnancy outcome and treatment durations for each age group, one for younger women and one for older women. As shown below, there were no significant relationships between pregnancy outcome and treatment duration, either within younger women ($\chi^2(1) = 0.86, p = .353$), or within older women ($\chi^2(1) = 0.37, p = .545$).

Table 13. Comparison of Pregnancy Outcomes by Treatment Durations

(< 3 Months vs. > 3 months) within Age Groups (35-39 vs. 40-47)

<i>Age group</i>	<i>Treatment duration</i>		<i>Pregnancy</i>		<i>Total</i>
			<i>Yes</i>	<i>No</i>	
35 to 39	< 3 months	Count	8	5	13
		%	61.5%	38.5%	100.0%
	3+ months	Count	9	11	20
		%	45.0%	55.0%	100.0%
Total		Count	17	16	33
		%	51.5%	48.5%	100.0%
40 to 47	< 3 months	Count	6	10	16
		%	37.5%	62.5%	100.0%
	3+ months	Count	5	13	18
		%	27.8%	72.2%	100.0%
Total		Count	11	23	34
		%	32.4%	67.6%	100.0%

<i>Age</i>		<i>Value</i>	<i>df</i>	<i>p-value</i>
35 to 39	Pearson Chi-Square	0.86	1	.353
40 to 47	Pearson Chi-Square	0.37	1	.545

3. Effects of TCM Treatment Durations on Pregnancy Outcome and the Effects of Concurrent Western Fertility Treatment Use

Out of the 67 patients in this study, 35.8% (N=24) patients received ART, IUI, and/or COH treatments in conjunction with TCM therapy while 64.2% (N=43) patients received TCM therapy only and no other western medical fertility treatments (see Table 14 below).

Table 14. Concurrent use of Western fertility treatments and TCM therapy

		<i>N</i>	<i>Percent</i>
<i>Concurrent Use of Western Fertility treatments with TCM Therapy</i>	<i>Yes</i>	24	35.8%
	<i>No</i>	43	64.2%
<i>Total</i>		67	100.0%

Table 15 below further presents the types of Western fertility assisting procedures used in conjunction with TCM therapy. The most common types of procedures used was ART (50.0%) and secondly IUI (29.2%).

Table 15: Types of Western fertility treatments used with TCM therapy

	<i>N</i>	<i>Percent</i>
<i>Treatment types</i>	<i>IVF only</i>	12 50.0%
	<i>IUI only</i>	7 29.2%
	<i>FET only</i>	1 4.2%
	<i>Mixed (IVF, IUI, COS)</i>	4 16.7%
<i>Total</i>	24	100.0%

In order to rule out the effect of Western medical treatments that might be skewing the pregnancy results, two parallel chi-square analyses were conducted to see if there might be a difference in pregnancy outcome based on treatment duration for those who received TCM therapy only as compared to those who received TCM plus Western medical procedures. As shown in Table 16, Chi square analysis yielded no significant relationships between pregnancy outcome and treatment duration, either within those who received TCM treatments alone ($\chi^2(1) = 1.31, p = 0.347$), or within those who received both TCM combined with Western medical procedures ($\chi^2(1) = 0.20, p = 0.653$).

Table 16: Comparison of Pregnancy Outcomes by TCM Treatment Durations (< 3 Months vs. Longer) within Treatment Types (TCM Only vs. TCM plus Western fertility treatments)

<i>Treatment type</i>	<i>Treatment Duration</i>	<i>Pregnant</i>		<i>Total</i>	
		<i>Yes</i>	<i>No</i>		
<i>TCM + Western fertility treatments</i>	<i>< 3 months</i>	Count	4	3	7
		%	57.1%	42.9%	100.0%
	<i>3+ months</i>	Count	8	9	17
		%	47.1%	52.9%	100.0%
<i>Total</i>		Count	12	12	24
		%	50.0%	50.0%	100.0%
<i>TCM treatments only</i>	<i>< 3 months</i>	Count	10	12	22
		%	45.5%	54.5%	100.0%
	<i>3+ months</i>	Count	6	15	21
		%	28.6%	71.4%	100.0%
<i>Total</i>		Count	16	27	43
		%	37.2%	62.8%	100.0%

		<i>Value</i>	<i>df</i>	<i>p-value</i>
TCM + Western medical procedures	Pearson Chi-Square	0.20	1	.653
TCM only	Pearson Chi-Square	1.31	1	.252

Optional Exploratory Analyses

Optional exploratory analyses were engaged to study the effects of TCM treatment durations on live birth outcomes of AMA women with infertility. Though not the main outcome measured for this study, live birth outcome is an important outcome for assessing the viability and effectiveness of fertility treatment thus these exploratory analyses were engaged.

1. Live Birth Outcomes

a. Effects of TCM Treatment Durations on Live Birth Outcome

A comparison of live birth outcome by treatment duration is presented in Table 16 below. A Fisher's exact test of live birth outcome (Yes vs. No) did not reveal a significant difference by length of treatment ($p = 0.690$). Fisher's exact test was used as an appropriate analysis since this was a comparison of two dichotomous (two-category) variables with the cell sizes that were smaller than 5. Fisher's exact test to compare the live birth outcome by treatment durations within age groups (younger group age 35- 39 vs. older group age 40-47) also did not reveal a significant difference by length of treatment ($p=1.0$ vs. $p=0.49$ respectively) (see Table 18 below). Furthermore, a comparison of live birth outcome by TCM treatment durations within treatment types (TCM only vs. TCM + Western treatments) was analyzed. Fisher's exact tests revealed no significant difference on live birth outcome by lengths of treatment ($p=1.00$ vs. $p=1.00$ respectively) (see Table 19 below). There were unknown outcomes of live births ($n=14$) because those patients did not continue with TCM treatments after pregnancy.

Table 17: Comparison of Live Birth Outcomes by TCM Treatment Durations
 (< 3 months vs. > 3months)

Treatment Duration		<i>Live Birth</i>		<i>Total</i>
		<i>Yes</i>	<i>No</i>	
< 3 months	Count	2	19	21
	%	9.5%	90.5%	100.0%
3+ months	Count	5	27	32
	%	15.6%	84.4%	100.0%
Total	Count	7	46	53
	%	13.2%	86.8%	100.0%

Fisher's exact test, $p = 0.690$

Table 18. Comparison of Live Birth Outcomes by TCM Treatment Duration

(< 3 months vs. > 3 months) within Age Groups (35-39 vs. 40-47)

<i>Age groups</i>	<i>Treatment duration</i>		<i>Live Birth</i>		<i>Total</i>
			<i>Yes</i>	<i>No</i>	
35 to 39	< 3 months	Count	2	7	9
		%	22.2%	77.8%	100.0%
	3+ months	Count	3	12	15
		%	20.0%	80.0%	100.0%
Total		Count	5	19	24
		%	20.8%	79.2%	100.0%
40 to 47	< 3 months	Count	0	12	12
		%	0.0%	100.0%	100.0%
	3+ months	Count	2	15	17
		%	11.8%	88.2%	100.0%
Total		Count	2	27	29
		%	6.9%	93.1%	100.0%

<i>Age</i>		<i>p-value</i>
35 to 39	Fisher's Exact Test	1.00
40 to 47	Fisher's Exact Test	0.49

Table 19. Comparison of Live Birth Outcomes by TCM Treatment Durations within Treatment Types (TCM +Western tx group vs. TCM only group)

<i>Treatment type</i>	<i>Treatment Duration</i>	<i>Live Birth</i>		<i>Total</i>	
		<i>Yes</i>	<i>No</i>		
TCM + Western fertility treatments	< 3 months	Count	1	4	5
		%	20.0%	80.0%	100.0%
	3+ months	Count	3	11	14
		%	21.4%	78.6%	100.0%
<i>Total</i>		Count	4	15	19
		%	21.1%	78.9%	100.0%
TCM treatments only	< 3 months	Count	1	15	16
		%	6.3%	93.8%	100.0%
	3+ months	Count	2	16	18
		%	11.1%	88.9%	100.0%
<i>Total</i>		Count	3	31	34
		%	8.8%	91.2%	100.0%

Treatment type	p-value
TCM only	Fisher's Exact Test 1.00
TCM + Western fertility treatments	Fisher's Exact Test 1.00

2. Assessing Adverse Effects and Additional Benefits of TCM Therapy

Toxicity and adverse effects of TCM therapy especially the CHM have always been a concern by conventional medical community as well as the public (Wing, 2009). In order to initiate the effort of assessing the safety and adverse effects of TCM therapy, adverse effects reported by patients are presented below (see Table 20 & 21 and Illustration 9 below). 9% ($N=6$) of the patients reported adverse effects from their TCM treatments either from herbal medicine or acupuncture while 91% ($N=61$) of the patients reported no adverse effects during their TCM therapy. The most common complaint reported was bloating from herbal therapy, and other complaints included gas, diarrhea, and stomachache from taking Chinese herbs. These symptoms were short term effects and were resolved immediately after their herbal prescriptions were adjusted. The only adverse effect reported from acupuncture in this study was pelvic pain. This section of the study only reports the data available from the medical charts in order to provide a general view regarding adverse effects of TCM therapy. Therefore, further analyses on this subject were not in the scope of this study.

Table 20. Adverse effects (yes or no) reported during TCM therapy

		<i>f</i>	%
Adverse Effects Reported	Yes	6	9.0%
	No	61	91.0%
Total		67	100.0%

Illustration 9. Adverse effects (yes or no) reported during TCM therapy

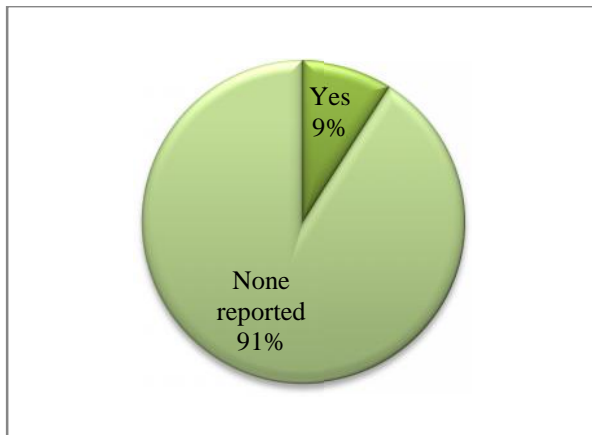


Table 21: List of Adverse Effects Reported during TCM Therapy

		<i>f</i>
Adverse Effects Reported	Bloating from herbs	3
	Gas from herbs	1
	Diarrhea from herbs	1
	Stomachache from herbs	1
	Pelvic pain after acupuncture	1
Total		7

Positive experiences reported by patients during their TCM treatments are presented below (see Table 22 and Illustration 10 below). These data are analyzed in order to assess the patients' experience during TCM therapy along with assessing additional benefits of TCM therapy. 34.3% ($N=23$) of patients reported positive effects during TCM therapy while no positive remarks were mentioned in 65.7% ($N=44$) of the patients in this study. Table 23 presents commonly seen positive effects reported by the patients in this study. Out of the positive effects reported (34%) the common effects include amelioration of low back pain and joint pain

(22.4%) and improvement in ovulation and menstrual period related symptoms, such as alleviation of dysmenorrhea and improvement in regularity of ovulation and period (18.4%) (see Table 23 and Illustration 11 below). Other effects include improved energy (8.2%), improved mental and psychological state (8.2%), amelioration of insomnia (8.2%), and decrease in constipation (8.2%) (See Table 23 and Illustration 11 below). This section of the study only reports the data available from the medical charts in order to provide a general view regarding positive effects reported during TCM therapy in this study. Therefore, further analyses on this subject are not in the scope of this study.

Table 22. Positive Experience (yes or no) Reported during TCM Treatments

		<i>f</i>	%
Positive Experience Reported	Yes	23	34.3%
	No	44	65.7%
Total		67	100.0%

Illustration 10. Positive Effects (yes or no) Reported during TCM Treatments

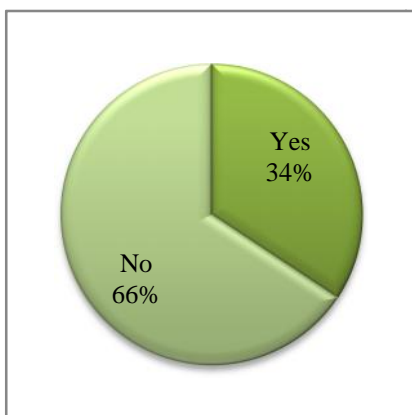


Table 23. Positive Experience Reported during TCM Treatments

	<i>f</i>	%
Decreased low back pain and joint pain*	11	22.4%
Improved period/ovulation related symptoms**	9	18.4%
Better energy	4	8.2%
Better mental/psychological state***	4	8.2%
Insomnia improved	4	8.2%
Decreased constipation	4	8.2%
Decreased night sweats	3	6.1%
Others****	10	20.4%
Total	49	100.0%

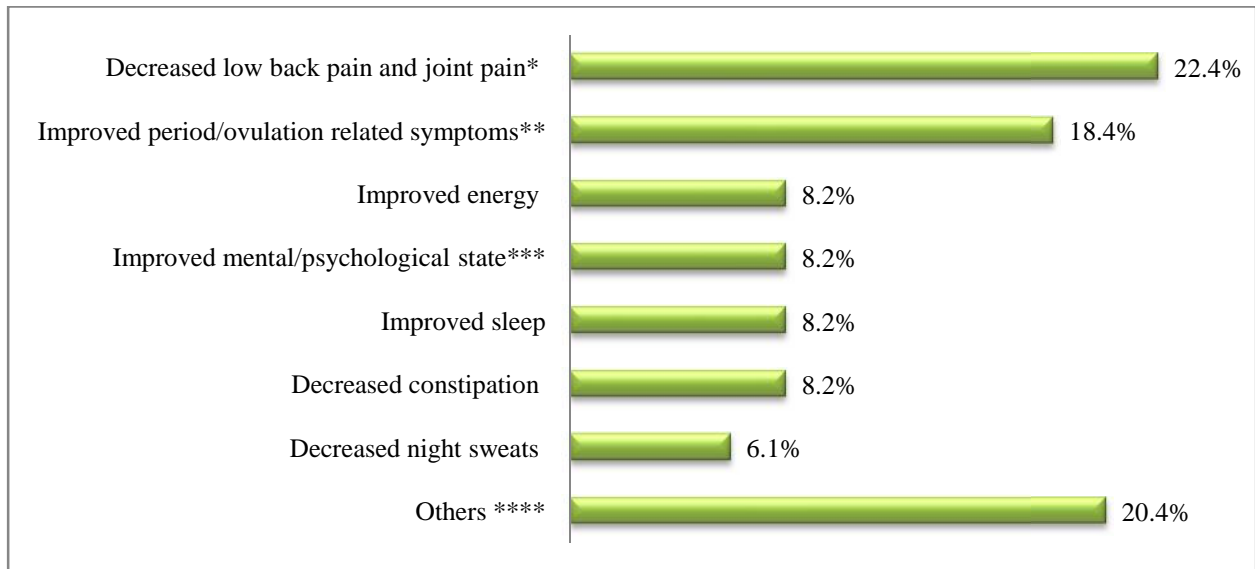
* Include knee pain, wrist pain, and sciatica.

** Include decreased dysmenorrhea, improved PMS, improved regularity of ovulation, improved regularity of period, improvement in libido, and improvement in vaginal lubrication

*** Include depression, anxiety, and irritability

**** Include allergy, headache, psoriasis, palpitation, hypertension, nocturia, chills, cold hands/feet, weight loss, increased follicle counts, and increased fertilization rates

Illustration 11. Positive Experience Reported during TCM Treatments



* Include knee pain, wrist pain, and sciatica.

** Include depression, anxiety, irritability

*** Include decreased dysmenorrhea, improved PMS, improved regularity of ovulation, improved regularity of period, improvement in libido, and improvement in vaginal lubrication

CHAPTER 5: DISCUSSION

Summary of Findings

This study investigated the effects of different TCM treatment durations (1- < 3 months vs. > 3 months) on pregnancy outcomes (yes or no) of infertile AMA women. A retrospective chart review was engaged as the research design for this study. 67 patients' charts were chosen as eligible and were studied out of more than 500 charts reviewed from an established TCM clinic specializing in TCM infertility treatments in Santa Monica, CA, USA. The hypothesis of this study was that TCM treatment durations of more than 3 months would yield to better pregnancy outcome than the treatment duration of less than 3 months. This hypothesis was based on the clinical experience of the investigator as well as the observations from the available TCM literatures and the folliculogenesis.

The result of this study revealed no significant difference on the pregnancy outcomes (yes or no) between the different durations of TCM therapy (1- < 3 months vs. > 3 months). TCM therapy duration of 1 to less than 3 months (n=29) yielded to 48.3% pregnancy rate (n=14) while the duration of more than 3 months (n=38) yielded 36.8% pregnancy rate (n=14). A Pearson Chi-Square analysis revealed no significant statistical difference ($p=0.347$) between the pregnancy rates of the two duration groups. Though not statistically significant, there is a trend seen that the TCM treatment duration of 1 month to less than 3 months yielded to a slight higher pregnancy rate than the longer treatment of more than 3 months (48.3% vs. 36.8% respectively).

Furthermore, in order to rule the age effects that may be skewing the results, further analysis was engaged. The patients were divided into two groups by the median split for age, which was age 40. One group consisted of patients aged 35-39 (n=33) and the other consisted of patients aged 40-47 (n=34). A Pearson Chi-Square analysis revealed no significant difference on

pregnancy outcomes (yes or no) between the two durations (1- < 3 months vs. > 3 months) within the two age groups. The group of age 35-39, the duration of 1 month to less than 3 months yielded 61.5% pregnancy rates while the duration of more than 3 months yielded to 45.0% pregnancy rates which demonstrated no significant difference ($p = 0.353$). The group of age 40-47, the duration of 1 month to less than 3 months yielded 37.5% pregnancy rates while the duration of more than 3 months yielded to 27.8% pregnancy rates which demonstrated no significant difference ($p=0.545$). Overall, the pregnancy rate was higher among the younger age group (age 35-39) for both duration groups (1- < 3 months and > 3 months).

Another important factor to rule out was the effects of Western medical fertility assisting treatments that may be skewing the pregnancy outcomes (yes or no). 35.8% of patients ($n=24$) in this study underwent some form(s) of Western medical fertility treatments such as IVF, IUI, or controlled ovulation stimulation while undergoing TCM therapy. Two parallel chi-square analyses were conducted to see the difference in pregnancy outcomes based on TCM treatment durations (1- < 3 months vs. > 3 months) among patients who received TCM therapy only compared to those who received TCM therapy plus Western fertility treatments. The results revealed that there were no significant differences on pregnancy outcomes between the two treatment duration groups (1- < 3 months vs. > 3 months) within TCM only group and TCM plus Western fertility treatment group (45.5% vs. 28.6%; $p= 0.252$ and 57.1% vs. 47.1%; $p=0.653$ respectively). Though not statistically significant, the data appeared to indicate a trend that integration of TCM therapy and Western fertility treatment yielded to a higher pregnancy rates than TCM only treatment group in both duration groups.

Additional exploratory analyses were engaged to determine the effects of TCM durations on live birth outcomes (yes or no) of AMA women with infertility. Though the live birth

outcome was not the main outcome measured for this study, it is an important outcome in order to assess viability and efficacy of fertility treatment thus this exploratory analyses were engaged. The live birth outcomes (yes or no) of patients in the treatment duration group of 1 month to less than 3 months (n=29) had 6.9% (n=2) live birth rate while patients in the treatment duration group of more than 3 months (n=38) had 13.2 % (n=5) live birth rate. The Fisher's exact was engaged to see the effects of TCM durations on the live birth outcome. The results revealed no significant difference by length of treatment (1- < 3 months vs. > 3 months) ($p = 0.69$). Though not statistically significant, TCM treatment durations of more than 3 months yielded higher live birth outcome than the treatment duration of less than 3 months. (13.2 % s. 6.9% respectively).

The age effects on the live birth outcomes within the two treatment duration groups (1- < 3 months vs. > 3 months) were also analyzed. The patients were grouped into two age groups by median split of age, which was age 40 (age 35-39 vs. age 40-47). Within the age group of 37-39 (n=24) the treatment duration of 1 month to less than 3 months (n=9) yielded to 22.2% live birth rate (n=2) while the treatment duration of more than 3 months (n=15) yielded to 22.0% live birth rate (n=3). A Fisher's exact test for the two duration groups (1- < 3 months vs. > 3 months) within this age group revealed no significant difference on live birth outcome ($p=1.0$). Within the age group of 40-47 (n=29), the treatment duration of 1 month to less than 3 months (n=0) yielded to 0.0% live birth rate (n=0) while the treatment duration of more than 3 months (n=17) yielded to 11.8% live birth rate (n=2). A Fisher's exact test for the two duration groups (1- < 3 months vs. > 3 months) within this age group revealed no significant difference on live birth outcome ($p=0.49$). Though not statistically significant, overall live birth rates were higher among the younger age group (age 35-39) in both treatment duration groups (1- < 3 months vs. > 3 months). Moreover, though statistically not significant, there is a small trend seen that the live

birth rate among the older age group (age 40-47), the live birth rate was slightly higher among the longer treatment duration of more than 3 months.

The effects of concurrent Western fertility treatment(s) (yes or no) within the two TCM treatment durations (1- < 3 months vs. > 3 months) on live birth outcomes (yes or no) were also analyzed. Patients were separated into two groups: one group consisted of patients who underwent TCM therapy only and no Western fertility treatments (n=43) and the other consisted of patients who underwent Western fertility treatments during their TCM therapy (n=24). The effects of TCM treatment durations (1- < 3 months vs. > 3 months) on live birth outcomes (yes or no) within these two treatment groups were analyzed. In TCM only group, the treatment duration group of less than 3 months (n=21) yielded 6.3% live birth rate (n=1) while the treatment duration group of more than 3 months (n=21) yielded 11.1% live birth rate (n=2). In the TCM plus Western fertility treatment group (n=24), the treatment duration group of less than 3 months (n=7) yielded 20.0% live birth rate (n=1) while the treatment duration group of more than 3 months (n=17) yielded 21.4% live birth rate (n=3). For both treatment groups (TCM only vs. TCM + Western fertility treatment), there were no significant differences found from Fisher's exact tests ($p=1.00$ and $p=1.00$ respectively) to indicate the effects of different treatment durations on live birth outcome. Although not statistically significant, there was a trend that the treatment group of TCM therapy plus Western fertility treatment yielded slightly higher live birth rates in both duration groups (20.0% in 1- < 3 months group and 21.4% in > 3 months group) than TCM only group (6.3% in 1- < 3 months group and 11.1% in > 3 months group). The results consisted of unknown outcomes for live birth in this study. This was because that the many pregnant patients did not continue the TCM treatments so there were no records indicating the live birth outcomes of the patients.

Implications for Theory

The purpose of this study was to compare the effects of different TCM treatment durations on pregnancy outcomes of AMA women with infertility. This study initiated a new area of research regarding effective durations of TCM therapy to achieve pregnancy among infertile AMA patients. To my best knowledge there are no studies that have investigated this particular topic among AMA patients. Despite the lack of literatures, studies that investigate effective treatments and treatment methods are in need because of increasing AMA patient population and limited treatment options available for this patient population. However, the literature mentioned in the earlier chapters that have demonstrated the efficacy of TCM therapy for improving pregnancy outcomes and related reproductive functions have become the foundation of the current study. Wing (2009) has demonstrated the efficacy of CHM therapy for improving pregnancy outcome and physiological parameters involved in reproduction. This study yielded 34% pregnancy rate after 3 menstrual cycles of CHM among patients who have had at least 2 failed IVF cycles previously. The pregnancy rate demonstrated in the current study after 3 months of TCM treatment was 36.8% which was similar to the pregnancy rate demonstrated by Wing (2009). Unlike the study by Wing (2009), patients in the current study used acupuncture as well as Chinese herbal medicine. Addition of acupuncture treatment to Chinese herbal therapy in this current study may be a positive factor contributing to pregnancy rates of 48.3% with TCM treatment duration of less than 3 months. From this observation, the combination of acupuncture and CHM appears to be a better treatment method for improving pregnancy outcome than using CHM alone. With the combination therapy of acupuncture and CHM therapy, AMA patients may be able to achieve pregnancy faster than using CHM alone as a treatment. The median age of women in the study by Wing (2009) was age 42, which was

similar to the median age of the current study, which was age 40. The current study also included patients who have a history of failed IVF (median of 2 cycles) as well as failed IUI (median of 3 cycles). The current study confirms the benefit of TCM therapy for patients with history of multiple failed IVFs and other western fertility treatments. Although unproven, this finding may initiate a foundation for establishing TCM therapy as an effective alternative therapy for AMA patients whose prognosis for IVF is often poor as well as for patients who have had failed IVF cycles.

Implications for Practice

From the findings of the current study, it is reasonable to state that AMA patients are likely to respond to TCM therapy within the first 3 months of treatments. Although not statistically significant, this trend from the current study implies that the 3-month period may be considered as an appropriate treatment cycle for TCM therapy when treating AMA patients with infertility. However, when AMA patients continue with TCM treatments longer than 3 months, it appears to have no adverse effects on pregnancy outcomes but pregnancy results were seen among the AMA patients studied in this review. Furthermore, though not statistically significant, there was a trend seen that TCM treatments that are longer than 3 months may increase live birth outcomes of AMA patients more than the less than 3-month treatment duration especially among patients who used TCM therapy only or among the older AMA patient group (age 40-47). However, no such trend was seen among younger AMA women of age 35-39 and patients who have used combination of TCM therapy and Western fertility assisting treatments. Therefore, treatment duration of longer than a 3-month period can be beneficial and appropriate, and patients should be educated that treatments beyond 3-month is not necessarily an indication of negative outcome for pregnancy.

Though not statistically significant, data from the current study also have indicated a positive trend of combining TCM therapy and Western medical fertility treatments yielding to slightly higher pregnancy outcomes and slightly higher live birth outcomes than the TCM therapy only group in both duration groups. This finding may indicate the benefit of integration of TCM therapy and Western fertility treatments as well as the importance and the need for the collaboration between TCM practitioners and Reproductive endocrinologists and gynecologists especially for the management and the treatment of age related infertility. Patients should be educated by both TCM and Western medical professionals about possible beneficial outcome of combining TCM therapy and Western fertility treatments when appropriate.

Furthermore, from demographic statistical data, this study found that the median age of advanced maternal aged women seeking for TCM therapy was 40 and the median months patients spent trying to conceive prior to seeking TCM therapy was 18 months. These findings suggest for TCM practitioners the need to improve public education about possible benefits of TCM therapy for patients in order to increase awareness and knowledge about treatment options available before patients spend months of trying to conceive without successful outcome. Since 43.3% of the patients in this study had prior history of multiple failed Western medical fertility treatments such as IVF, IUI, and COS, it is important that Reproductive Endocrinologists to be aware of and educate the possible benefits of TCM therapy as a valid, effective therapy for AMA patients with history of unsuccessful outcomes. This is especially important among AMA patients because of their progressively declining fecundity and limited treatment options.

One of the factors leading to a small sample size of this study was that many patients were not eligible for this study because of lack of regularity and consistency in their acupuncture treatments and intake of CHM prescribed. This finding implies the need for TCM practitioners to

educate patients better about regular treatments and intake of CHM and the need to encourage the regularity of treatments.

The adverse effects reported by the patients in this review were minimal and easily managed, and there were various positive experiences reported by the patients during the TCM therapy in this review. Though this is only a preliminary finding and only indicates short term effects, reproductive endocrinologists, gynecologists, and other Western medical professionals as well as TCM practitioners should be aware of these positive aspects of TCM therapy and educate patients.

Limitations of the Current Study

There are threats to internal and external validity in this study. Threats to internal validity are confounding factors including many variables existed in the sample used for the current study. These variances include Western and TCM diagnoses, variance in acupuncture and herbal treatments administered, variance in the practitioners who provided treatments, variance in treatment frequencies, variance in medications used for ART and IUI, different frequencies or combinations of ARTs, IUIs and COS and how TCM was incorporated with during ARTs, IUIs, and COS.

Although more than 500 charts were reviewed, only 67 charts were eligible for this study leading to a small sample size. A small sample size was another major threat to external validity and lead to the results to be not statistically significant. Because this study was retrospective chart review that used medical charts that were recorded not for the purpose of a research, there were possibility of unrecorded data or unreadable data which became a threat to internal validity of the study.

Purposive, convenient, non-random sampling method used for this study includes sampling bias that undermines the external validity of the current study and limits the findings of this study as generalizable for the population. Therefore, the limitations of this study are summarized as confounding factors and weakness in generalizability of the findings. Nonetheless, the purposive sample of current study provided a valuable opportunity for examining data pertinent to a sample of advanced maternal aged women who sought fertility treatment.

Despite the limitations and weakness of the current study, the study served as a pilot study, initiating a new area of research and established a foundation that provides direction and guidance for future research.

Recommendations for future research

For the future research, a study with a larger sample size is recommended to improve the statistical significance of the data. Furthermore, in order to limit confounding factors and improve validity of the study, a prospective study is recommended. Standardizing inclusion criteria such as Western medical diagnoses associated with infertility, TCM diagnosis, as well as standardizing herbal formula and acupuncture treatment will help to reduce confounding factors as well. However, there is a dilemma that since TCM therapy is based on an individualized and customized therapy for efficacy, standardizing treatments may limit the effectiveness of TCM treatments, which can become a limitation in conducting a randomized controlled trial for TCM therapy.

More future studies indicating the mechanisms, benefits, and safety of acupuncture and Chinese herbal medicine for treating infertility are needed to demonstrate evidence based efficacy and safety of TCM therapy as a valid treatment for infertility. Studies demonstrating

effective integration of TCM therapy and Western fertility treatments such as ARTs are also needed to confirm the positive trend seen for pregnancy outcomes of AMA patients in this study.

Conclusion

The current study found that there was no statistically significant difference between a shorter TCM treatment (1 month to less than 3 months) and a longer treatment (more than 3 months) on the pregnancy outcomes of AMA patients with infertility. Successful pregnancies were seen among patients in both treatment durations groups. Since time is a stressful factor among AMA patients with infertility because of the inevitable, progressive decline of fecundity among AMA patients, having this knowledge on the effects of TCM treatment durations on pregnancy outcome will help educate the patients more clearly about TCM treatments and reduce their anxiety and stress about TCM therapy.

The efficacy and benefits of TCM therapy for improving pregnancy outcomes and improving reproductive related functions have been established by various authors. The current study further contributes to add evidence based information about an appropriate method and administration of TCM therapy to the existing evidence. Furthermore, this study initiated a new area of study involving AMA patients with infertility and also an effort to establish an effective application of TCM therapy, which no current studies have yet to determine and investigate to the scale of current study. This pilot study should benefit as a foundation for future research in the area of AMA related infertility or studies indicating the efficacy and safety of TCM therapy.

This study also demonstrated a positive trend of integration of TCM therapy and Western fertility assisting treatments, which contributes to initiate more collaboration and integration of TCM practitioners and Reproductive endocrinologists and other Western medical professionals for the benefit of AMA patients and improving their treatment outcomes.

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APPENDIX 1 -- Chart Abstraction Log

Appendix 1. **Chart Abstraction Log**

Patient age/Occupation	Age: ____ Occupation: _____ Name: _____ ID: _____
TGP how many years:	
Previous history of pregnancy, miscarriage, abortions, and Live birth	unknown: ____ # of Live birth: ____ # Miscarriage(s): ____ #of Abortion(s): ____ Miscarriage #1 @ ____ wks, Miscarriage #2 @ ____ wks Miscarriage #3 @ ____ wks, Miscarriage # 4 @ ____ wks
Prior use of ART > 3 months prior to TCM treatment	____ Yes ____ No ____ Unknown # of cycles/# of pregnancy: IVF ____/____ GIFT: ____/____ ZIFT: ____/____ FET: ____/____ Stimulated IUI ____/____ Fertility drugs ____/____
Concurrent use of ART with TCM	____ Yes ____ No ____ Unknown # of cycles: IVF: ____ GIFT: ____ ZIFT: ____ FET: ____ Stimulated IUI: ____ Fertility drugs only: ____
Treatment duration in months until pregnancy wit or last period	____ Treatment duration until pregnancy (B-hCG test, ultrasound): ____ days ____ # of acu txs ____ No pregnancy: ____ days ____ # of acu txs ____ Unknown
Western DX	Endocrine-ovulatory Tubal Uterine Cervical-Pelvic Autoimmune Others Unknown
TCM Dx	
Acupuncture used	
Herbs used	
Positive or adverse effects reported	
Live birth post TCM tx or combination of TCM & ART	____ Yes ____ No ____ Unknown
ART performance	# of eggs retrieved # of eggs fertilized # of embryos transferred Unknown
	Cycle 1
	Cycle 2
	Cycle 3
	Cycle 4
	Cycle 5

APPENDIX 2 -- Data Collection Log

Appendix 2. Data Collection log

Group A-a: TCM + Western tx < 3 months

Patient ID	Preg 1=yes 2=no	Age	Months TGP	# of Prior preg	# of Prior Live births	# of prior abort.	# of prior misc.	Prior ART, IUI, &/or COS use 1=yes 2=no	Concurrent ART, IUI &/or COS	Live birth post tx 1=yes 2=no 3=unknown	Days until preg or LMP	West. DX	TCM DX	Adverse effects	Positive remarks	Acu pts	herbs used
A-a-1																	
A-a-2																	
A-a-3																	
A-a-4																	
A-a-5																	

Group A-b: TCM + Western tx > 3 months

Patient ID	Preg 1=yes 2=no	Age	Months TGP	# of Prior preg	# of Prior Live births	# of prior abort.	# of prior misc.	Prior ART, IUI, &/or COS use 1=yes 2=no	Concurrent ART, IUI &/or COS	Live birth post tx 1=yes 2=no 3=unknown	Days until preg or LMP	West. DX	TCM DX	Adverse effects	Positive remarks	Acu pts	herbs used
A-b-1																	
A-b-2																	
A-b-3																	
A-b-4																	
A-b-5																	

Group B-a: TCM tx only < 3 months

Patient ID	Preg 1=yes 2=no	Age	Months TGP	# of Prior preg	# of Prior Live births	# of prior abort.	# of prior misc.	Prior ART, IUI, &/or COS use 1=yes 2=no	Concurrent ART, IUI &/or COS	Live birth post tx 1=yes 2=no 3=unknown	Days until preg or LMP	West. DX	TCM DX	Adverse effects	Positive remarks	Acu pts	herbs used
B-a-1																	
B-a-2																	
B-a-3																	
B-a-4																	
B-a-5																	

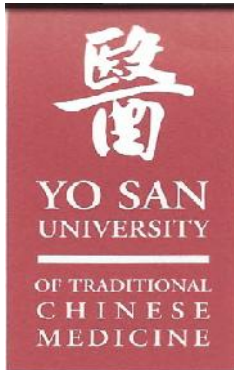
Group B-b: TCM tx only > 3 months

Patient ID	Preg 1=yes 2=no	Age	Months TGP	# of Prior preg	# of Prior Live births	# of prior abort.	# of prior misc.	Prior ART, IUI, &/or COS use 1=yes 2=no	Concurrent ART, IUI &/or COS	Live birth post tx 1=yes 2=no 3=unknown	Days until preg or LMP	West. DX	TCM DX	Adverse effects	Positive remarks	Acu pts	herbs used
B-b-1																	
B-b-2																	
B-b-3																	
B-b-4																	
B-b-5																	

ATTACHMENT 1—IRB Approval Letter

Yamamoto Capstone

Attachment 1—IRB Approval letter



April 25, 2012

Kumiko Yamamoto, L.Ac.
10206 Regent St #12
Los Angeles, CA 90034

Dear Kumiko,

Your Claim for Exemption from the Institutional Review Board (IRB) has been reviewed. Your research proposal has been approved, with no recommendations effective April 1, 2012 through March 31, 2013.

Should there be any significant changes that need to be made which would alter the research procedures that you have explained in your proposal, please consult with the IRB coordinator prior to making those changes.

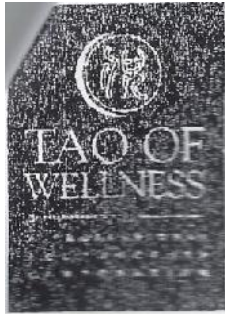
Regards,

Debra Jean Rawdin
IRB Coordinator

13315 West Washington Boulevard, Los Angeles, CA 90066 Tel 310.577.3000 Fax 310.577.3033 www.yosan.edu

ATTACHMENT 2-- Authorization Letter from Tao of Wellness Clinic

Attachment 2 -- Authorization letter from Tao of Wellness Clinic



March 23,2012

Yo San University
IRB/Capstone Thesis
13315 W. Washington Blvd.
Los Angeles, CA 90066

Re: Authorization to Use Patients' Chart Notes

To Whom It May Concern:

This letter is written to confirm that Kumiko Yamamoto LAc. has authorization from Adriana Rivera, Operations Manager, to use patients' medical chart notes for the capstone/thesis research project. The collected patients' information must abide by all HIPAA standards. Patient's identity and other private information will not be released for this project, and must remain confidential at all times.

If you should have any further questions, please do not hesitate to contact me directly at 310-917-2200 ext. 239. Thank you.

Sincerely,

Adriana Rivera

Operations Manager

Tao of Wellness

310.917.2200 ext. 239