

# Unexplained Recurrent Pregnancy Loss and the Psycho-Neuro- Immunologic Network: Immuno-modulation with Traditional Chinese Medicine and other CAM Modalities

A Capstone Project

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## **Abstract**

The purpose of this study was to expose the effects of TCM on the immune system and the possible benefits of TCM therapies in the treatment of unexplained recurrent miscarriage, either as part of an integrated treatment plan or on its own. Using research synthesis method, data from 34 articles were compiled regarding the ability of Traditional Chinese Medical (TCM) treatments, (herbal medicine and acupuncture), along with other complementary and alternative modalities, (taiqi, qi gong and yoga), to change immunological profiles in immunologically-related diseases, during a stress-response, and in miscarriage.

The data were initially abstracted in an article abstraction form and then analyzed into several tables. The data revealed that herbs, acupuncture, and other CAM therapies do indeed have effects on the immune cells that are implicated in the pro and anti-inflammatory states responsible for disease and miscarriage. However, the effects of herbs, acupuncture, and other CAM therapies did not appear to consistently promote changes in the direction that is thought to favor pregnancy or minimize the risk for pregnancy loss. As this is a preliminary study, the data were discussed in consideration to the limitations of the studies. The lack of randomization and blindedness, partially due to the difficulties of randomizing and blinding samples for ethical reasons, encourage the need for future research with sufficient sampling, with the hopes of finding clinically useful treatments, applicable to the population of couples who struggle with repeated pregnancy losses.

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## **Chapter I: Introduction**

### **Background**

Miscarriage can imply genetic damage. Only an estimated 30% of all human conceptions result in a viable embryo, and 50% of those are lost even before the start of the next menstrual cycle (Choudhury & Knapp, 2000). Ninety percent of fetuses with malformations are spontaneously aborted during early pregnancy and a reported sixty percent of first trimester spontaneous abortions have chromosome abnormalities, (“Environmental Causes for Infertility”, n.d.). There are numerous materials and foods now implicated in contributing to genetic malformations. For instance, it was reported that coffee drinking during, and even before pregnancy, was associated with over twice the risk of miscarriage when the mother consumed 2-3 cups of coffee per day (“Environmental Causes for Infertility”, n.d.). Chemical solvents found in nail polish and paint, neurotoxins such as aspartame and arsenic, as well as vaccinations, and even agave (Dr. Weil, 2008), are suspected to cause risks to the fetus in utero. According to renowned Chinese physician Dr. Guo Zi Qian (2011), the apparent increase in infertility rates are due mainly to male factor. Eggs have greater protection surrounding them and sperm do not. This makes sperm more susceptible to environmental stressors, such as vehicle emissions, chemicals in the air and water, combustion products, and pesticides. All these factors reduce sperm/egg bonding. Hence everyday substances come with a potential to negatively influence fetal development.

Miscarriage can also imply a defect in the maternal immune response. It was reported that eighty percent of unexplained recurrent pregnancy loss is due to an immunological component (Petranyi et al., 2001). An immune dysfunction during pregnancy may prevent the

woman's body from distinguishing between self and non-self. This would inhibit the uterus from protecting the growing ball of allogenic cells inside. The uterine immune environment has been recognized as a unique and privileged place (Magarelli, P, Yo San DAOM lecture, 2010).

The interface between the mother and the growing fetus has intrigued physicians and evolutionists alike since it is a phenomenon that the co-existence of different genetic backgrounds during pregnancy can survive the maternal immune system. Healthy fetal growth certainly requires a specific interaction between maternal cells and trophoblasts to form a placental unit. This happens when the maternal endometrium successfully accepts the foreign blastocyst during the timely implantation window (Mourik, Macklon, & Heijnen, 2009). There is also an assortment of cells in various concentrations that are active during normal and pathologic pregnancy. These include natural killer cells (NK cells), which are the predominant immune-cell population, and Th1 and Th2 cytokines, which include tumor necrosis factor, (TNF- $\alpha$ ), interferon- $\gamma$ , (IFN- $\gamma$ ), and interleukins. It is the delicate interaction, or balance, between these various immune cells that results in a successful pregnancy. However, their exact roles throughout the pregnancy period remain elusive in both eastern and western traditions.

Most of the literature concluded that increases in Th1 cytokines, and associated cells such as TNF- $\alpha$ , and IFN- $\gamma$ , and elevations in NK cells, create risks for the fetus and increase chances of miscarriage (Choudhury & Knapp, 2000; Kanellopoulos-Langevin, Caucheteux, Verbeke & Ojcius, 2003). However, there is disagreement over the relative predictive value of measuring each variable to determine subsequent miscarriage (Shakhar et al., 2006; Tuckerman, Laird, Prakash & Li, 2007). Because of the degree of unknown surrounding unexplained recurrent miscarriage, there are limited treatment options for women who experience repeated pregnancy

losses. During a conversation with Florence M. Hofman Ph.D, a professor of pathology and immunology at the Keck School of Medicine of USC, I expressed my confusion about the complexities of the cytokine network during immunologic pregnancy loss. She said that biomedicine has not yet found an effective treatment for this condition and that if beneficial and practical therapies exist to support these patients, they should be recognized. If Chinese medicine, (through individual pattern differentiation), could offer solutions to balance immune function towards a state more favorable for pregnancy, many couples would clearly benefit.

Experientially and historically the effectiveness of Chinese medicine in the treatment of miscarriage has been recorded in *The Complete Book of Effective Prescriptions for Diseases of Women* published in 1237 A.D.. Clinically we know Traditional Chinese medicine, (TCM), works for the treatment of reproductive disorders. This is evident as more Chinese medical practitioners choose to specialize in reproductive health and greater numbers of patients seek complementary or alternative treatments for their reproductive concerns because of positive treatment outcomes. There are at least a dozen conditions related to reproduction, especially infertility, which can be addressed with herbs and acupuncture. Chinese medicine can assist in ovulation induction in anovulatory polycystic ovarian patients, help restore the hypothalamic-pituitary-ovarian axis in menstrual disorders, or help decrease the intense pain and bleeding associated with endometriosis.

Infertility is defined specifically as the failure to conceive after a year of regular intercourse, (or 6 months if the woman is over 35), without the use of contraception. Miscarriage or spontaneous abortion falls under the category of infertility. For some couples with a history of miscarriage(s), Chinese medicine (herbs and acupuncture) may be the sole

treatment that produces a healthy baby. When the recurrent loss is not due to anatomic complications, Chinese medicine, particularly Chinese herbal medicine, can help balance the immune system during high risk pregnancies. Recurrent abortion is defined as three consecutive losses, (although that definition varies and may include two consecutive losses), and is associated with genetic, anatomic, and endocrine causes. Patients with allogeneic recurrent miscarriage do seek fertility treatments, such as ART, and struggling couples and physicians would benefit from discovering additional and potentially useful treatments that could prevent future losses.

This study is motivated by my own clinical observations and by a curiosity to better understand the relationships between Chinese herbal medicine, acupuncture, stress-reducing techniques like qi gong, taiqi, and immunological and stress-induced disease processes. This study will investigate the effects of chronic and acute stress on the immune system and in miscarriage. Through a comprehensive analysis of selected journal articles, reviews, and a case study, this paper will explore how the actions of TCM treatments and CAM therapies might moderate the immune system and stress responses which can upset immune balance and leads to miscarriage.

### **Research Objective**

Over the last year and a half, I have witnessed a handful of patients with previous pregnancy losses become pregnant and conceive healthy babies once under the care of an experienced TCM practitioner. Perhaps if more couples were aware of the possibility of how TCM can support pregnancy outcomes, there would be greater interest in acupuncture and herbal medicine and therefore more treatment choices. Also, miscarriage is unfortunately a very

common occurrence in the United States with reports that 1 in 4 or even 1 in 3 pregnancies will end in early termination (Schieve, 2003). Of those pregnancies that don't survive, fifty percent of them are unexplained.

Once anatomic, endocrine, or other causes are ruled out, doctors might suspect fetal rejection by the maternal immune system. Western treatment options for intolerance at the maternal-fetal interface can be limited and are impractical, expensive, and are still experimental (Opinion, 2006; Tang, Alfirevic & Quenby, 2011). Therefore a primary motivation behind this research synthesis is to expose the possible benefits of TCM in the treatment of unexplained or allogeneic recurrent miscarriage or threatened abortion, and generate awareness about treatment options.

This study has three objectives.

The first is to offer practitioners an understanding of how Chinese herbs, acupuncture, and other CAM modalities, affect physiology in immune-mediated disease and miscarriage in western terminology. Basic knowledge of the inflammatory mechanisms and components of the immune system which are active during recurrent miscarriage is useful when therapies are integrated and require thoughtful communications with patients and western doctors.

The second objective is to increase awareness of Chinese medicine in the treatment of miscarriage. This is accomplished by critical analysis of previous studies that support the use of Chinese herbs to treat allogeneic pregnancy loss.

Finally, immunological issues are intimately connected to stress, which makes treatment of the nervous system crucial. It is my intention to elaborate on the connection between acute

and long-term psycho-emotional stress and immune deregulation. The ability of acupuncture to reduce mental stress through an inhibition of the sympathetic nervous system has been recognized (Middlekauf et al., 2002). This paper will explore the possibility of how stress-induced immunological miscarriage may be a candidate for Chinese medical therapies.

This study will systematically research whether TCM and CAM can affect RPL. It is my intention to shed light on whether my clinical experience is backed by previous studies and to inform couples who are eager to conceive, but fearful of enduring subsequent losses, that they might have options for effective care that are financially practical. If the application of TCM extends to the treatment of immunological miscarriage, this study may provide western doctors with a vital perspective on the best ways to support patients who may benefit from integrative therapies if necessary.

### **Statement of Research Questions**

The primary questions associated with this project are:

1. What are the effects of Chinese herbal medicine, acupuncture, and other CAM modalities in the treatment of immune-mediated diseases?
2. To what degree can these treatments help couples successfully conceive who have previously experienced unexplained recurrent miscarriage? More specifically; can Chinese herbal medicine, acupuncture, and other CAM modalities, support the immunological parameters necessary to promote live birth in women with a history of miscarriage, such as a shift towards TH2, or a reduction in inflammatory cells in women with elevated levels?

My hypothesis is that actions of specific Chinese herbs, acupuncture, and CAM therapies can modulate the maternal immune response during pregnancy in a way that reduces risk of miscarriage. By mediating certain immune responses, these treatments can promote a balance between the pro and anti-inflammatory cytokines that are necessary to support a healthy pregnancy.

## **Chapter II: Literature Review**

### **Overview**

Certain concepts will be covered in this project including:

- The mechanism which permits foreign cells to survive inside the mother in a normal pregnancy, or the mechanism behind the pathological maternal immune response to fetal tissue during miscarriage
- Western medicine's current treatment options for miscarriage
- The effects of Chinese medicine and other CAM modalities on the immune system and uterine immune cell populations
- The immune cell functions that are enhanced or depressed during stressful life events,
- The connection between stress factors and recurrent pregnancy loss, and
- The possibility of a relationship between traditional Chinese medicine and immunological or stress-related pregnancy loss.

### **Resources Engaged**

Current medical information regarding immunological pregnancy loss was gathered from published studies or journal reviews. This was accomplished primarily by searching online journal articles in PubMed and Human Reproduction. Key immune variables were identified that are thought to be responsible for immune-mediated miscarriage. These included, Th1 and

Th2 cytokines, NK cells, IFN- $\gamma$  and TNF- $\alpha$ . Since one purpose of this project was to elaborate on the connection between stress and immune function, the impact of stress on these cells was investigated and will be discussed in the following sections.

### **Causes of Miscarriage - Suspected Immune Imbalances**

There are two main explanations for recurrent pregnancy loss. The first is that a chromosomal abnormality is preventing the embryo from developing properly. This is confirmed by karyotyping from the partners, or by pre-genetic determination in assisted reproductive technologies. The second is that problems exist within the uterine environment that do not allow for proper implantation and fetal development. Certain cellular immune factors are necessary to secure the birth of a healthy baby. If a woman experiences multiple losses for which no cause can be identified, doctors might suspect immune system involvement. Complex interactions between cytokines, lymphocytes, and other factors are critical for immune system maintenance and regulation, (Figure II). Some of the suggested mechanisms for recurrent loss include the presence of cytotoxic Th1 cells, (Figures I, and III) and alterations in natural killer cell, (NK cell), frequency and cytotoxicity in the decidua and periphery (King, Smith, Chapman & Sacks, 2010). NK cells are innate immune cells that account for 5-15% of peripheral lymphocytes (Arck et al., 2000). There are a host of other variables involved in miscarriage and RPL including maternal blocking antibodies, (Choudhury, S. and Knapp, L., 2000), granulocyte macrophage stimulating colony (GM-SCF), and nuclear translocation factor kappa beta (NF- $\kappa$ B) (Wang, Yu, Yan, & Wang, 2011). Activation of NF- $\kappa$ B is thought to promote the formation of pro-inflammatory Th1 cytokines like IL-6, IL-8, and TNF- $\alpha$ .

In addition to cell-mediated immune responses, other genetic and immunologic parameters in spontaneous miscarriage patients include a variety of humoral immune components such as inappropriate sharing of the human leukocyte antigen (HLA), trophoblast lymphocyte cross-reactive, (TLX), (Belingard et al., 1995), decidual suppressor cells (Daya, Clark, Devin, Jarrel & Chaput, 1985), antipaternal cytotoxic antibodies (APCA) and mixed lymphocyte reaction blocking factor (MLR-Bf), (Agrawal, Pandey, Mandal, Mishra & Agarwal, 2002).

The above mentioned pro-inflammatory mediators are the potential targets for therapeutic strategies designed to inhibit inflammatory actions and reduce the chance of pathological tissue damage which would result in a nonviable birth. The challenge for biomedical doctors is to determine the significance or prognostic value of measuring these markers. Reproductive failure is a multi-factorial problem and measurements of cell frequency, or even activity, may not be representative of cell cytotoxicity or subsequent pregnancy outcomes (Quenby et al., 2009; Tuckerman, Laird, Prackash, & Li, 2007).

During normal pregnancy, the mother is exposed to a variety of potentially foreign antigens, on the ball of cells growing inside the uterine cavity. The extent to which her antibodies and cell-mediated immune responses play a role in the survival of fetal allograft is still not fully understood despite Western medicine's exceptional ability to treat reproductive conditions. According to the majority of immunological research, cells associated with the Th1 response, (IL-2, IL-3, IFN- $\gamma$ , TNF- $\alpha$ ), and NK cells are most frequently cited for their detrimental effects on the fetus during at-risk pregnancies. Therefore this paper will focus

primarily on these types of cells and the influence of Chinese medicine and other complementary treatments on these cellular inflammatory actions.

Besides the significant role played by these immune cells in allogeneic embryo loss in early pregnancy, they are also essential components of the immune system, implicated in immune-mediated disease, and influenced by stress (Clark et al. 2005; Elami-Suzin & Mankuta, 2007; Lissauer, Piper, Moss, & Kilby, 2009; Saito et al, 2007).

### **Stress and its Impact on Immune Imbalances and Inflammation**

Immunological functions are affected by psychosocial factors (Suguiura-Ogasawara, et al., 2002) as stress is known to disturb homeostasis and cause behavioral, endocrine, and immunological changes. For example, severe psychological stress (as in loss of a loved one), was demonstrated to cause significant alterations in normal immune function (Bartrop, Luckhurst, Lazarus, Kiloh & Penny, 1977). It is generally thought that the immune system is suppressed by chronic stress, depression, and anxiety (Hori et al., 2000) and if stress chronically overwhelms immune function, the body's innate defenses become skewed and disease is more likely (Kour et al, 2009). Alternatively, it is also suggested that the sympathetic nervous system does not simply suppress the immune system under stress, but instead it might help organize the activity and distribution of immune cells (Elenkov & Chrousos, 1999).

The type of stress, and its duration, is highly significant for predicting the course of immune-related disease, therefore it might be too general to assume that stress is immunosuppressive (Elenkov & Chrousos, 1999) since stress hormones are selective and will inhibit and activate immune cells to generate a hypo or hyperactive immune-related disease. Both trauma or acute stress and long-term stress can cause alterations in the activities of the

central nervous system. However, it is chronic stress, not acute stress, which encourages central nervous activity that stimulates a breakdown in immune function (Galinowski, 1993).

Depression especially down-regulates the immune system (Andalib et al., 2006). Increased numbers of white blood cells have also been associated with depression.

Stress in general has been associated with increased secretions of glucocorticoids and catecholamines. In order to restore equilibrium in the body, these substances inhibit IL-12, TNF- $\alpha$ , INF- $\gamma$ , but upregulate IL-10, IL-4, and TGF- $\beta$  production (Calcagni & Elenkov, 2006). When stress causes the release of inflammatory cells, there is corresponding activation of Th2 cytokines. This shift towards Th2 is to protect the organism from the flood of pro-inflammatory cells (Wyman et al., 2007). The body always tries to maintain homeostasis.

Glucocorticoid receptor (GR) function is also involved in the stress-immune relationship. GR levels are mediated by glucocorticoids and under stress, GR levels decrease. The result is a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent suppression of immunity (Dickens, Romero, Cyr, Dunn & Meddle, 2009).

According to Sugiura-Ogasawara et al. (2002) stress increases IL-1 $\beta$  and TNF- $\alpha$ , which are associated with the Th1 response and miscarriage. They also stated that stress reduces IL-2, INF- $\gamma$ , MHC class II and NK activity, which are all actions thought to promote pregnancy. They did not differentiate between chronic and acute stress. Depression is its own subset since it is specifically associated with INF- $\gamma$ , and IL-1 $\beta$ , elevation and a down-regulation of IL-2 and reduced NK cell activity (Sugiura-Ogasawara et al., 2002). Therefore stress and depression lower NK cell counts.

Inflammation in itself is an essential physiologic process that happens during both menstruation and pregnancy (Wang et al., 2010). Uncontrolled inflammation however is pathological. Cytokines are immune hormones that mediate and control immune and inflammatory responses (Calcagni & Elenkov, 2006). It is the interaction between cytokines such as TNF- $\alpha$ , IL-1, IL-2 and IFN- $\gamma$  during immune and inflammatory reactions that works to restore the imbalance triggered by an activation of the stress system.

Both the neuroendocrine and immune systems are involved in homeostasis maintenance. This means that therapeutic interventions geared towards the treatment of one will affect the other. Based on the above reasoning, the psycho-neuro-immune-endocrine network may be a proposed target in the prevention of immune dysfunction and immunological miscarriage. Stray-Pedersen and Stray-Pedersen (1984) even demonstrated that tender loving care can improve success rates in patients with unexplained habitual abortion (as cited in Sugiura-Ogaswara et al., 2002 p.2580). If analyses of the studies show that acupuncture, herbs and other CAM modalities can influence immune cells in cell-mediated and humoral responses, it is possible that emotional balancing may be a subsequent outcome of TCM treatments that are intentionally designed to impact immune function. Several basic tenets of physiology are assumed here. The first is that physiologic pregnancy requires healthy immune function. The second is that stress is immunosuppressive (Calcagni & Elenkov, 2006). Based on those assumptions, one can consider that stress will impact pregnancy outcomes.

### **Stress and miscarriage**

Psychological stress has been proposed to cause miscarriages as well as infertility (Andalib et al., 2006, Arck et al, 2001, Hori et al., 2000). Stress can be thought to be abortigenic

via the psycho-neuro-immunological pathways, (Arck et al. 2001). Not only may psychological stress influence pregnancy outcomes by elevated stress hormones that reduce fetal vascularization and oxygen supply (Andalib et al., 2006), but miscarriage itself is certainly a psychological trauma and depressive thoughts that persist post-abortion can influence hormonal and immunologic patterns (Andalib et al., 2006). The importance of hormones in mediating miscarriage is not discussed in this paper even though their significance is clearly central to the progression of pregnancy

Several authors agree that chronic stress in adults is consistently associated with decreases in NK cell cytotoxicity (Arck, et al., 2001; Andalib, et al., 2006; Wyman, 2007). A decrease in NK cell cytotoxicity is a favorable shift for patients with RPL who are thought to have excess NK cell activities. It is this down-regulation of NK cell cytotoxicity during chronic stress which seems to contradict the assumption that high NK cell levels are associated with stress which can also be associated with greater risk for miscarriage. An experienced Chinese medicine practitioner in the field of reproductive disorders expressed that measuring NK cell cytotoxicity may not be entirely relevant for predicting miscarriage (B. Horn Ph.D, 2011, personal communication). This might be because NK cells are necessary especially during early pregnancy. He also mentioned that there is a distinct subset of NK cells, known as uterine NK cells, which are thought to play a role in early pregnancy loss.

The degree of control which we have over our stressors may play a role in the severity of the immune response. Andalib et al. (2006), wrote that depression correlates with higher numbers and activity of circulating white blood cells in women with RPL but not with NK activity. He also wrote that depression does correlate with cytotoxicity which is the mechanism responsible for causing miscarriage. The relationship between pre-conceptual NK cell activity

and subsequent miscarriage seems to be inconsistent. Contradictory to what would be expected, Hori et al. (2000) wrote that pre-conceptual NK activity was negatively correlated with women's neuroticism and depressive symptoms but positively correlated with self-esteem. In other words, he reported that the higher one's self esteem, the higher is the NK cell activity. Counterintuitive as it may seem, perhaps the image of high self-esteem described is one of a woman who controls her emotions, holds a demanding job, and identifies more with her work than her emotional body.

As previously mentioned, unexplained miscarriage may be in part due to a Th1/Th2 cytokine imbalance at the feto-maternal interface. It is generally accepted that a shift towards Th2 favors pregnancy outcomes while one towards Th1 strongly correlates with pregnancy failure in mice and humans (Arck et al. 2000). Decidual or uterine natural killer (uNK) cells are also implicated and have been shown to release the Th1 cytokine IFN- $\gamma$  after stimulation with IL-12 and IL-2 (Hori, et al., 2000). Pregnancy and immunological functions are known to be under the influence of various psychological factors (Sugiura-Ogasawara et al., 2002). The research has not unanimously identified an immunological alteration with Th1/Th2 profiles and NK cell imbalances in the deciduas of recurrent aborters that correlates with perceived psychological stress. Stress can be defined as coping with environmental challenges and an associated activation of the neuronal and neurohumoral systems (Beszczynska, 2007). Though what qualifies as a challenge is unique to the individual. Chinese medical treatments made according to differential diagnosis may offer support that is specific to the needs of the patient and enhance the coping mechanisms.

## **Current Challenges for Western Medicine in Treating Miscarriage**

Western treatment options for RPL are limited. Once anatomic reasons are ruled out, typical treatment protocols include anti-coagulants, like aspirin or heparin, for patients with anti-phospholipid antibodies, and Metformin to reduce blood sugar for patients with insulin problems. If these treatments are unsuccessful or if there is no diagnosis indicating clotting or blood sugar problems, clinicians may recommend immunotherapy or IVIg. IVIg is made up of human-derived antibodies and given intravenously prior to conception all the way through until the sixth month. There are two theories that support the use of intravenous immunoglobulin (IVIg). The first is that the antibodies keep the immune system from identifying the fetus as foreign and attacking it. Second, IVIg is also thought to help minimize the actions of NK cells (King, Smith, Chapman, & Sacks, 2010). High levels of NK cells are thought to prevent implantation and interfere with proper development of the placenta which is thought to increase the chance of miscarriage (Dosiou & Guidice, 2005).

IVIg is administered intravenously which requires the insertion of catheters into a hand or arm vein to allow for a slow drip. This method can sometimes last up to several hours in length and is necessary in order to minimize the chances of unpleasant side effects like fever, chills, nausea, and various body pains (Opinion, 2006). Some sources report a success rate as high as 80% with IVIg after recurrent miscarriage, yet the dose and frequency of treatment is not clearly decided on (Malinowski, 2001). IVIg is expensive with each dose costing as much as \$1500. This means that receiving IVIg therapy during pregnancy could cost in excess of \$10,000. So in terms of practicality and expense, IVIg is a physical and financial burden. Considering all of the

above, the question remains whether an expensive and possibly detrimental treatment should be given without a proper diagnosis.

### **Chinese Medicine's Potential for Treating Stress**

As with any medical treatment, including Chinese medicine included, there is no guarantee for success and if improperly prescribed or performed, there is the potential to harm with herbs and acupuncture. However, under the supervision of a competent Chinese medical doctor herbs are completely safe. While IVIg is not without risk to mother and fetus, this type of treatment is reserved primarily for women with unexplained recurrent abortions or in cases where accepted or usual therapies failed (Constantinescu, Cozmei, Dumitrascu, & Carasevici, 2008).

Here is where Chinese medicine can contribute. The diagnosis for RPL in TCM is not simply RPL or unexplained RPL. TCM diagnosis is made according to the patient's pattern which includes all current signs and symptoms with consideration given to medical history, genetics, age, current lifestyle and dietary habits. A basic principle of TCM theory is that a patient is not the numbers on their lab results or their western diagnosis. The saying "same disease different pattern, different disease same pattern", certainly applies to RPL. Two women diagnosed with RPL who seek treatment with TCM, may be given different TCM diagnoses and therefore different treatments, (Jian, 1997). Likewise, one patient with RPL and one with chronic migraines, for example, may be given the same TCM diagnosis and roughly same treatment plan despite different Western diagnoses.

Clinically, treatment for patients with a history of multiple miscarriages will likely include herbal medicine and acupuncture, as well as the necessary lifestyle and dietary or supplement recommendations. I chose to include complementary and alternative medicine (CAM) modalities such as exercise and meditation in this paper since the practice of Chinese medicine is not simply limited to lying still on a table with needles and drinking herbal teas. Lifestyle, exercise, and nutritional prescriptions are considered part of the treatment plan and invaluable for patient progress and treatment success. While it was not possible to find a documented and reliable source which defines specific activities that prevent miscarriage for women with a previous history of loss, this project focuses on how certain therapies might affect the immune system via the nervous system to influence pregnancy outcomes. By mitigating the negative effects of nerves or stress on the body, the immune system can be wired in a way that promotes pregnancy and calms a “restless fetus”.

### **The Effects Chinese Medicine on Stress and Immune Modulation**

An important aspect of TCM’s success with RPL is its ability to modulate immune functions (Chen, 2010), as well as counteract any hormonal disturbances created by stress-induced states. The immune-modulating effects of acupuncture were explained in a review by Cabioğlu and Cetin (2008). Acupuncture is thought to affect the immune system via the release of endogenous opioid peptides. The authors explained that the anti-inflammatory benefits of needle stimulation happen when cells near the insertion site secrete substances such as adrenocorticotropic hormone (ACTH) and serotonin. These hormones cause vasodilatation which supports the release of cytokines, like TNF- $\alpha$ , IL-6. The release of these inflammatory cytokines stimulates the hypothalamic-pituitary-adrenal axis and the result is the secretion of

glucocorticoids which balance the inflammatory cascade through the secretion of anti-inflammatory cytokines like IL-10 (Cabioglu & Cetin, 2008).

According to Clark et al. (2005) stress is implicated in immunological pregnancy loss. An outstanding feature of TCM is that it is known to balance both the immune and nervous system and promote homeostasis (Cabioglu & Cetin, 2008; Novak et al., 2001; Wu et al., 2010; Yim et al., 2010). Since the immune and nervous system share responsibility in multiple body functions and are in constant communication with each other via the psycho-neuro-immune network, imbalances in one will ultimately impact the other. This is where the relationship between stress and well-being is valuable to recognize, and treatment of the mind-body is indicated. The previous section was a reminder of how intimately related those systems are.

### **Literature Review Integration**

It is still not fully understood whether unexplained recurrent miscarriage in women and spontaneous abortion in animal models is caused by failure of maternal immunoregulatory control or by non-immunological hormonal factors (Billington, 1989). The challenge of traditional allopathic medicine is to devise a proper treatment strategy for a diagnosis of miscarriage due to unknown etiology. Elevated NK cell numbers are thought to be present in the deciduas of recurrent aborters, but opinions differ (Shakar et al., 2004). It is also questionable whether or not the number of NK cells directly correlates to their cytotoxicity on the uterus during miscarriage (Gregory, et al., 1985). If effective treatment depends on the cause, a successful treatment should include a proper diagnosis.

The hypothesis for this study is that TCM and other CAM treatment modalities benefit women with previous miscarriage by moderating the immune response towards one that favors

pregnancy. If the synthesized data indicate that Chinese medicine does benefit the uterus and the immune system in general, then this information would offer complementary solutions for both patients and practitioners.

The literature shows that herbs, acupuncture, and other CAM modalities may be able to prevent miscarriage by balancing immune cells in a way which favors the specific conditions necessary to maintain a pregnancy.

### **CHAPTER III: Data Analysis**

This study will describe the methods that have been used for finding and analyzing quasi-experimental or qualitative studies that investigate the effects of herbal medicine, acupuncture and other CAM modalities on securing pregnancy.

#### **Research Objectives and Hypothesis**

Research synthesis methodology was chosen in order to explore several objectives. The first objective was to investigate the effects of Chinese herbs, acupuncture, and other CAM modalities on immune cells in various disease states and when the body is disrupted by stress. The second objective was to specifically examine the immuno-modulatory actions of Chinese medicine in women with unexplained miscarriage. The third objective was to emphasize the relationship between stress, anxiety and depression and the physiological changes in the neuro-endocrine and immune systems, and if and how these changes might specifically impact reproductive outcomes.

In order to create a hypothesis, PubMed was searched for publications relating to “the effects of Chinese herbs and acupuncture on immunological miscarriage”. Search terms included immune, immunity, immunological AND herbs, herbal, Chinese herbs AND acupuncture AND miscarriage, pregnancy loss, abortion. CAM modalities and identified immune cells were added to the search to address the immuno-modulatory effects of therapies like qi gong and taiqi. These initial searches revealed certain herbs and formulas known for promoting pregnancy and reducing miscarriage. Therefore these specific herbs or formulas, (like

Zhuyun III or Tu Si Zi), were searched with specific cytokines (like Th1/Th2, NK cells, IFN- $\gamma$ , etc.), and combined with miscarriage, abortion, and pregnancy loss.

Both inductive and deductive analysis approaches were used in this project. Patterns or themes were extracted from articles. The patterns identified led to the tentative hypothesis that Chinese medicine can effectively treat recurrent pregnancy loss or threatened abortion through immune-modulating mechanisms.

Based on clinical experience and through inductive reasoning, the hypothesis was generated that Chinese medicine and CAM modalities can promote changes in immune cells in ways that reduce the risk of miscarriage.

### **Research Design and Rationale**

This is a preliminary study with broad parameters. The primary goal is to reveal themes for the purpose of further exploration in later systematic studies. This is not a complete analysis of research findings because it is simply descriptive. In this method the researcher's epistemological stance is objective. The rationale for choosing a research synthesis was made for primarily one reason. Due to the nature of the research problems, this was the only doable type of project for the questions being asked given time and resources. A qualitative or descriptive method of analysis is the most appropriate for this topic. Qualitative method is also the most effective way to decipher the similarities and differences of themes and discover disparities among authors.

The most appropriate method for this research study was a systematic review of the relevant publications that involved quantitative studies that pertained to the hypothesis.

Systematic review is especially useful in the analysis of data which originate from randomized controlled trials. However, it is a challenge for trials using Chinese medical treatments and especially acupuncture, to design and implement a placebo treatment. For this study, only two of the studies found were randomized and controlled. Every attempt was made to employ names for themes from actual words of the data and then group the themes in manner that directly reflect the text as a whole.

Personal biases were minimized in this study. Since I am aware that I am partial to Chinese medicine in the treatment of miscarriage whose success I have observed in the clinic, I made extra efforts to be critical and thorough. In order to limit the potential impact of personal feelings or subjective thoughts, the main research tool engaged was sorting, naming, or labeling themes as they appeared in the analyzed publications. This was accomplished by an article abstraction form which is not included in this paper for reasons due to length. The uncovered themes are discussed in chapter five.

### **Data Collection**

Data collection began in March 2010 and ended in September 2011. Literature searches were mainly performed on PubMed, Human Reproduction, High Wire, and Google Scholar. Additional search sources included MdLinx OB/Gyn alert emails, Liebertine online and the listed references in applicable articles. The related citations that appeared at the right-hand side of the PubMed screen were continuously screened for related and relevant articles.

In order to gain a starting point and basic understanding of what the immune profile during unexplained immunological miscarriage might look like, the initial search on PubMed

was “("recurrent miscarriage" OR "pregnancy loss")AND (immunology OR immune)”. Over 900 relevant articles were retrieved. Titles and abstracts related to the topic of this study were scanned and search terms narrowed down to: (“unexplained miscarriage” OR “recurrent pregnancy loss” OR “habitual abortion” OR “pregnancy wastage” OR “spontaneous miscarriage” OR “recurrent spontaneous abortion”) AND (immunological OR immune OR immunology). The intention was to distinguish certain immune markers implicated in unexplained miscarriage.

Data were collected from articles, journal reviews, online sources, case studies, text books, and direct verbal transmission. Purposive sampling was used to identify sources that met the criteria for this study. The criteria for initial selection included all resources that covered topics related to immunological variables (immune cells) implicated in recurrent pregnancy loss according to sources in Western Medicine. Immunological variables were identified from these articles, searched in combination with TCM and CAM treatments, and selected for analysis.

Due to the small number of articles or resources available, exclusion criteria had to be limited. Therefore articles analyzed in this research synthesis included publications since 1970, animal studies, Chinese journal articles, and studies done based on a small sampling size. These limitations will be taken into account in the discussion of results. Articles were excluded when they described miscarriage due to known immunological etiology or when a western diagnosis other than unexplained recurrent miscarriage was given. Examples of such diagnoses are the presence of anti-thyroid antibodies or anti-cardiolipin antibodies.

After collecting data on biomedicine’s perspective of unexplained recurrent miscarriage, which were reviewed in the Literature Review chapter, this project was narrowed down to

certain concepts that related to the therapeutic strategies in TCM and CAM, to address changes in immune cells responsible for miscarriage.

### **Data Collection Instruments and Procedures**

Initial searches were performed to gain a basic understanding of changes in the immune system during RPL and changes in the immune system during stress. The following data collection process involved gathering articles with topics covering immunology, reproduction, miscarriage, and stress-related immune response in response to TCM and CAM interventions. After scanning the relevant abstracts and articles produced from the initial search on PubMed, key immune variables implicated in unexplained recurrent miscarriage were identified. They included but are not limited to the Th1 and Th2 cytokines, NK cells, IFN- $\gamma$  and TNF- $\gamma$ . Separate searches were performed on each variable in combination with pregnancy loss and all its permutations. This search proved very valuable to the research question since the purpose was to identify local and systemic changes in during miscarriage.

The next step was to connect these independent immune marker variables with Chinese herbal medicine, acupuncture, and CAM modalities, combined with miscarriage. All relevant articles were included in this study. Also included were three abstracts written in Chinese and published in the *Zhongguo Zhong Xi Yi Jie He Za Zhi* Journal. With the exception of one case study, there were no publications found for acupuncture related to miscarriage and the immune markers. Searches on CAM therapies including yoga, taiqi, or qi gong retrieved no articles when combined with miscarriage search terms.

When “miscarriage” was removed from among the search terms relating to immunology and TCM or CAM, the number of retrieved articles increased. This search was performed to link Chinese medicine and other CAM modalities to changes in the immune cells in order to identify the effects of Chinese herbs, acupuncture, and other CAM modalities on the concentrations of certain immune cells implicated for RPL.

One objective of this project was to investigate consequences of stress on the immune system. Therefore a search was performed in PubMed, High Wire and Google Scholar for articles relating to stress and its associated concepts of grief, depression and anxiety combined with independent variables associated with immunologically-related miscarriage such as NK cells, Th1/Th2 or cytokines. The initial search revealed over 4000 articles. Information from six of those articles were included in the Literature Review section

### **Data Organization**

From all the selected articles, the relevant information was abstracted. The primary data collection instrument was an article abstraction form which summarized key themes pertaining to this project. These included:

1) The influence of Chinese herbal medicine on immune cells during disease, during stress, and during miscarriage

2) The influence of acupuncture on immune cells during disease, during stress, and during miscarriage, (implantation), and

3) The influence of other CAM modalities, (qi gong, taiqi, yoga, recreational-music-making) on the immune cells thought to be implicated in miscarriage.

After searching the online data bases, there was only one case study which fit the inclusion criteria for acupuncture and miscarriage prevention. A search on PubMed for Chinese herbal medicine and miscarriage revealed 76 results but only eleven fit the criteria for this project. At this point, the research question was then expanded to include CAM therapies.

Data from the selected articles were extracted organized and displayed in a series of data collection tables, or matrices, to bring together and compare the results of primary research. The purpose was to systematically synthesize a broad range of topics such as immunology and miscarriage, the impacts of stress on the immune system, the effects of TCM and CAM treatments on the immune system, the effects of Chinese medicine and CAM on stress, and the effects of TCM and CAM on miscarriage, pertaining to this project.

The most reasonable and appropriate qualitative method for this project was a thematic content analysis that descriptively presents the data in a textual way. This project portrayed the contents of data by indentifying common variables and themes in the text, analyzing their frequency counts, and accounting for varying opinions amongst authors. From the text a list of common themes was extracted in order to illustrate the rate of recurrence and cohesiveness of themes across the authored texts. Table III shows the prevalence or frequency of immune cellular markers in biomedical articles as they are thought to contribute to pregnancy loss or miscarriage. Table III plots independent variables that contribute to miscarriage such as NK cells, cytokines, Th1/TH2 balance, inflammatory cells or oxidative stress against Chinese herbal treatments, acupuncture and CAM modalities. Table II plots independent variables and themes against text

sources to determine frequencies and/or dissimilarities. Independent variables included maternal lymphocytes like Th1/Th2 cytokines, natural killer cells, interleukin 1 and 2, progesterone, and prolactin. Relevant themes associated with this topic included “Chinese medicine may promote a shift away from Th1 and towards Th2 to support pregnancy” or “Huang Qin can increase serum progesterone”.

### **Detailed Method for Thematic Analyses**

The data were thematically analyzed in several stages. Articles were organized into three groups with subcategories. Group 1 related to the effects of Chinese herbs on a) the immune system, b) stress, and c) miscarriage. Group 2 related to the effects of acupuncture on a) the immune system, b) stress, and c) miscarriage/(implantation). Group 3 related to the effects of CAM therapies other than TCM such as qi gong, taiqi, yoga and recreational music-making on the immune system. The purpose was to collect data on the influence of these treatments on the immune cells suspected in causing miscarriage. This is essentially an exploration about cytokines or cellular immune modulators, their relationship to abortion and stress and their modulation by TCM and CAM.

## Chapter IV: Results

### Data Overview

In searching the online databases, roughly 200 citations were identified for initial consideration. After screening based on inclusion criteria, (topics covering abortion, immunology and TCM) and exclusion criteria, 34 studies including 5 abstracts remained for final content analysis. The search terms and resources used in this project were discussed in the previous section.

### Characteristics of the Analyzed Studies

#### Topics of Studies

Three groups of articles relating to the effects of herbs, acupuncture, and other CAM modalities, on immunological parameters, both in pregnancy-related conditions and other inflammatory conditions were analyzed (see below).

<b>Group</b>	<b>Number of articles (n)</b>
<b>Group 1, (the effects of Chinese herbs on immune markers in disease, stress, and miscarriage) of which:</b>	<b>21</b>
<b>1a</b> Chinese herbs and immunology	8
<b>1b</b> Chinese herbs and neuro-endocrine stress dysfunction	2
<b>1c</b> Chinese herbs and immunological miscarriage and abortion rates in IVF cycle	11
<b>Group 2, (the effects of acupuncture on immune cells, stress mechanism, and miscarriage/implantation during pregnancy) of which:</b>	<b>8</b>
<b>2a</b> acupuncture and immunology	5
<b>2b</b> acupuncture and stress	1
<b>2c</b> acupuncture and miscarriage/implantation	2
<b>Group 3a (the effects of CAM-taiqi, qi gong, yoga, and recreational music-making on the immunological profiles in human subjects)</b>	<b>5</b>

The notion that stress is a culprit in immunologically-related diseases and is involved in the pathogenesis of miscarriage is discussed in the literature review section.

### **Subjects**

The subjects in the analyzed articles consisted either of human subjects (n=16 articles), human cells in vitro (n=8 articles) or animals (n=10 articles). Of the seven studies which included in vitro human cells, all were in the herbal category, six were in the group relating to herbs and immunological imbalances, 1A, and one was in group related to herbs and miscarriage, 1C. The studies performed on humans and animals were distributed evenly throughout the groups.

### **Intervention Modalities**

Three different methods of herbal delivery were included as herbal treatment interventions. These were a single Chinese herb (n=1 article), isolations of herbal compounds (n=8 articles), or combinations of two or more herbs (n=9 articles). Two of the articles about Chinese herbs and their effects on immune cells were abstracts and did not identify the form of herbal delivery. The remaining studies included in this project used acupuncture or electro-acupuncture (n=8 articles), taiqi (n=1 articles), yoga (n=1 article), music (n=1 article), and qigong (n=2 articles).

### **Trial Sizes and Designs**

Among the 34 articles which documented experimental trials, one was single blinded, (Joos, Schott, Zou, Daniel & Martin, 2000) and two were randomized, (Joos et al., 2000; Wachi, Koyama, & Utsuyama, 2007). Group 1A consisted of herbs and their immunological influences

and included a total of eight articles, and one abstract (Hidaka et al., 1997). Six of these studies were carried out with human blood or cell samples. There was one animal study (Wu et al. 2010), and one which included seven healthy human volunteers (Hidaka et al., 1992). The treatment intervention was either an herbal isolate in six of the studies, an herbal formula in one study (Chen et al., 2004) or an unaltered herb in another study (Hidaka et al., 1992).

Articles in Group 1B were concerned with the effects of herbs on markers associated with the stress mechanism and included two articles which described the effects of chronic stress tests on rats. The studies performed stress tests for 180 minutes per day for seven or 21 days and 120 minutes per day for 30 days respectively. The effects of Chinese herbs on stress response were determined by measuring glucocorticoid receptor levels.

Articles in group 1C were concerned with the ability of Chinese herbs to change immunological parameters, or other pregnancy-related variables, in pathological states that correlate to miscarriage. The exception was the study by Liu and Wu (2006) which included 247 women undergoing IVF without previous miscarriage. The authors examined the efficacy of herbs on preventing miscarriage in IVF cycles. For this reason this study was included in this group.

Seven of the eleven trials included in group 1C (effects of herbs on miscarriage) used human subjects. A total of 268 women with threatened abortion, or with a history of recurrent spontaneous abortion, were included in six of these trials. Four studies were performed on animals, mainly mice. One study was carried out with cell cultures of human decidual cells. The size of the trials ranged from 34 to 247 participants. In the article by Liu and Wu (2006) the impacts of Chinese herbs on hormone changes and ultrasound were measured. The article by

Zhang et al. (2004) is included in this category because the fetal-maternal interface is similar to a graft-host interaction.

Group 2A relates to acupuncture's influence on the immune system and includes five articles. Two of these articles discussed trials performed on rats. Two studies discussed trials with human subjects with 20 and 34 participants respectively. One article discussed the use peripheral blood samples. Two of the five articles specified electro-acupuncture as the treatment intervention on rat models as opposed to acupuncture alone.

Group 2B covered acupuncture's effects on stress response and is limited to one article with 95 human subjects being treated with electro-acupuncture.

For group 2C, searches were performed to investigate the effects of acupuncture treatment alone on miscarriage and two articles were found. The first article by Huang, Zheng, Wu, and Wang (2010) discussed the role of acupuncture on improving blastocyst implantation using rat models. The second article was a case study by Standford, (2009), demonstrating the successful use of acupuncture and additional therapy in the treatment of a recurrent pregnancy loss patient with allergies and inflammation.

The articles in Group 3A related to complementary treatment modalities that are not TCM. Such treatment interventions included qigong, yoga, taiqi and recreational music making (drumming). All studies were done with human subjects. The trial size ranged from 19 to 37 participants.

### **Efficacy of Treatments on Changing Cytokine Profiles**

In all but two studies at least one immune variable or stress hormone was changed due to TCM or CAM treatment. The two exceptions were the study by Liu and Wu (2006) which measured hormone levels and used ultrasound to gauge the effects of an herbal decoction, and the study by Ushiroyama et al. (2006) which measured the number of days before uterine bleeding stopped, and the number of days for the retroplacental hematoma to disappear in women diagnosed with threatened miscarriage. Isolates of herbs, herbal formulas, single herbs, acupuncture and other complementary therapies either increased, decreased or had no effect on the variables being measured. The conditions studied in these articles included inflammatory conditions as well as pregnancy-related conditions. Some of the inflammatory conditions studied were rheumatic pain, allergies, bronchitis, and skin conditions. Each requires a different balance in immune markers to promote symptom relief. Therefore treatment efficacy is not reduced to a specific directional change in concentrations of cells. For trials done in group 1C, the efficacy of the treatment is measured by a shift in a direction known to sustain a healthy pregnancy. There are no outcome measures which include live birth.

### **Frequency of Variables across TCM and CAM treatment modalities**

The most frequently mentioned variable among the 34 articles was IL-10 (n=11), followed by IL-4 (n=7), and NK cells, TNF- $\alpha$ , and IFN- $\gamma$  (n=6). Next was IL-6 (n=5) and IL-2 (n=4). NF- $\kappa$ B, progesterone, CD4<sup>+</sup> lymphocytes, and blocking anti-bodies, and beta-endorphins were measured in 3 studies. Nitric oxide, mast cells, Th1, macrophages, CD3<sup>+</sup>, CD8<sup>+</sup>, prolactin and glucocorticoids were measured in two studies. The remaining variables

(retroplacental hematoma, bleeding, GM-CSF, CD57, oxidative stress, implantation and cortisol), were measured in only one study (see Table II).

### **Comparison of Changes in Independent Immunological Markers Independent of Treatment Modality**

Six studies in the 34 articles discussed NK cells measurements, (frequency and cytotoxicity). All six found NK cells to increase with treatment. A greater number of studies found TNF- $\alpha$  and IFN- $\gamma$ , which associated with miscarriage risk, to increase rather than decrease. All interleukins (IL-2, IL-4, IL-6, and IL-10) decreased more often than they increased, regardless of their function as pro or anti-inflammatory cytokines. The exception was the anti-inflammatory IL-8 which increased in one study but was not affected by treatment in another study.

The remaining pregnancy or miscarriage related independent variables, such as progesterone, prolactin, GM-CSF, and, blocking antibodies were all found to increase more than decrease. Glucocorticoid receptor (GR) levels were modulated in the rat models. One study demonstrated the abilities of an herbal formula to prevent a decrease in cytosolic GR levels and increased GR levels in the hippocampus (Mizoguchi et al., 2006). The other study demonstrated how an herbal formula can maintain the GR levels in chronically stressed rats (Chen et al., 2005). Macrophages and CD8<sup>+</sup> decreased in two of the studies. CD4<sup>+</sup> increased in one study and decreased in two others. Beta-endorphins increased in two studies and decreased in another. NF- $\kappa$ B increased in one study and decreased in two. Nitric oxide and mast cells both increased in one study but decreased in another. TH1 lymphocyte subsets were found to decrease in one

instance and have no change in another. Retroplacental hematoma, bleeding, CD57, oxidative stress, and cortisol were included in one study and all were found to decrease.

The effects of herbs on immune cells differed according to the herb being studied since each herb has different properties and functions in Chinese medicine, (hot, cold, moving, nourishing), and Western medicine, (anti-inflammatory, anti-viral, enhancing hormonal production). Acupuncture's effects on the different markers were fairly consistent. NK cells and TNF-a increased while interleukin concentrations decreased. This was the pattern in all studies except for Yamaguchi et al. (2007) which reported an increase in IL-4. Table III which summarizes the total counts and directional change of each variable independent of treatment, shows how the trends of interleukin concentrations are similar after both herbal and acupuncture treatments. NK cells, IFN- $\gamma$ , and TNF-a, which are considered detrimental to pregnancy were increased after acupuncture treatment.

## **Chapter V: Discussion**

### **Summary of Findings**

Unexplained recurrent miscarriage is thought to be caused by alterations in the immunological network which governs the implantation process via changes in pro-inflammatory and anti-inflammatory mediators, or in other words, by an imbalance between Th1 and Th2 cytokines and associated markers like NK cells. According to the selected and analyzed studies, certain herbal formulas and two herb combinations or herbal extracts were able to modulate the immune system and reduce the amount of pro-inflammatory cytokines thought to be responsible for early fetal loss and increase the amount of cytokines which support the implantation process and secure pregnancy. Studies on acupuncture and CAM treatments did not show a consistent reduction in pro-inflammatory cytokines or were not specifically addressing pregnancy-related conditions.

The data were collected and organized into several results tables. These tables demonstrated that Chinese herbal therapy, acupuncture and other complementary therapies such as taiqi, qigong, yoga, and recreational music making were able to cause changes in either direction of concentrations of immune cells and other variables implicated in miscarriage, and moderate inflammatory processes in human and animal subjects.

The western resources incorporated in this project concluded that changes in immune cells which contribute to inflammation are involved in miscarriage. However, their value in predicting subsequent miscarriage is questionable (Choudhury & Knapp, 2000; Shakar et al., 2006; Tuckerman et al., 2007). For this reason, it is difficult to say with certainty whether or not specific changes in immune cells will result in miscarriage.

One aspect of the hypothesis in this study was that stress contributes to immune system dysfunction. The data collected would indicate this is the case. Another aspect of the hypothesis was that herbal medicine and acupuncture mediate immune cells in a way that prevents immunological miscarriage, through a relative increase in anti-inflammatory and decrease in pro-inflammatory cytokines. According to the results, this was not consistently the case. Acupuncture and herbs can also increase inflammation and activate the immune system for instance by increasing INF- $\gamma$  and pro-inflammatory cytokine IL-2 (Carniero et al., 2010 Yamaguchi et al., 2007) which is associated with risk for miscarriage. .

### **Implications for Theory**

A major purpose of this project was to gather information and use deductive reasoning to discern whether the hypothesis that TCM and CAM treatments modulate the immune system in recurrent pregnancy loss patients is valid. An additional goal was to expose the clinical benefits of Chinese medicine in the treatment of abortion to the Western medical community. This was accomplished by moving from a broad generalization, (Chinese medicine treats abortion), towards specific observations, (Huang Qin can increase IL-10), through data collection and the analysis of contents. The intention was to acquaint patients and Western doctors with potentially complementary solutions to RPL in order to offer better treatment since standard immunotherapy protocols are not always successful.

To prepare the uterus for healthy implantation, a local inflammatory response is necessary. Inflammation is physiological and responsible for key processes during a woman's reproductive cycle which are accompanied by alterations in levels of female sex hormones. Menstruation, ovulation and the luteal phase of the cycle can induce systemic changes in

circulating levels of immune cells and pro-inflammatory cytokines (Yassin , Nasr, and Khalil, 2008) and cause a rise in body temperature and migration of cells towards the uterus.

While some inflammation is healthy, an over-exaggerated immune response due to excessive inflammation is not. It is generally accepted that the expression of pro-inflammatory TH1 cytokines (IL-2, IFN- $\gamma$  and TNF- $\alpha$ ) leads to pregnancy failure, while a shift towards TH2 cytokines (IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13) is thought to promote pregnancy (Ma et al., 2009). In contrast Wilczynski (2005) wrote, ‘both (Th1 and Th2) activities (are) inseparable like yin and yang. So the paradigm of “Th1-Th2 cooperation” is much closer to reality than “Th2 phenomenon”’. Th1 activity is important during the peri-implantation period and during premature and term labor, and can even surpass Th2 activity (Wilczynski, 2005). It would appear then that healthy pregnancy requires the immune system to be dynamic.

The material reviewed demonstrated that herbs, acupuncture and other CAM therapies were able to impact immune function and alter levels of pro and anti-inflammatory cytokines. It would be inaccurate to suggest that TCM only supported immune function in the direction that is typically thought to favor pregnancy. Chinese herbs (Li, WEn, Zhang, An, &Liu, 2011) and acupuncture (Arranz, Guayerbas, Siboni & De la Fuente, 2007; Yamaguchi et al., 2007) can also raise NK cells or pro-inflammatory cytokines, which are implicated in miscarriage. Part of the initial hypothesis was that TCM modulates the immune system through an individualized treatment according to the unique pattern of the patient. According to the findings, the therapeutic actions of TCM may play a role in mediating miscarriage in women who suffer from recurrent losses by modulating the immune system through differential diagnosis and individual care.

### **Implications for practice**

The roots of recurrent pregnancy loss (RPL) can be ambiguous simply because reproductive failure is multi-faceted. In other words, while the proposed etiology underlying unexplained RPL has an immunological origin, the specific contribution of each type of immune cell is not entirely clear. Because of this, therapy is not evidence-based. There is currently no specific treatment offered by Western medicine proven to be effective for the treatment of RPL (Dosiou & Giudice, 2005). The majority of the literature reviewed agreed that failure of the maternal immune system to support embryonic survival comes from inappropriate activity in the cytokine network at the fetal-maternal interface (Choudury & Knapp, 2000, Guerin, Prins, & Robertson, 2009, Savion et al., 2000). The reason for the need of this study was that the potentially immune-modulating effects of Chinese medical treatments and other complementary therapies may be able to assist couples who struggle with multiple pregnancy losses by modulating individual immune profiles towards a direction that favors full-term pregnancy. This may be achieved by supporting the production of the appropriate cytokine network. This project illustrates the abilities of TCM to change concentrations of immune cells and reduce the likelihood of subsequent miscarriage through immune system modulation. Since 80% of the immune system lives in the gastrointestinal tract, immune-imbalanced patients may benefit from acupuncture treatments directed at improving digestive health. Such treatments may include acupuncture or electro-acupuncture at ST 36 (Wang et al., 2009; Yamaguchi et al., 2007)

### **Limitations of the current study**

The factors that may limit the impact of the findings of this project present themselves on two levels. First, the validity of the analyzed studies themselves must be considered since many include animal subjects and small sample sizes. Only one study out of 34 was a randomized clinically controlled trial. The possibility of individual author bias of Chinese articles should also be considered. Secondly, there is the possibility that my personal biases may have influenced the way I collected and interpreted the data since I am the only rater. However, as doctoral level students, there has been a considerable amount of research methodology training in order to limit threats to internal validity and reliability. This risk was also minimized by the use of an article abstraction form

Treatment of patients according to individual pattern diagnoses is an important feature of Traditional Chinese medicine. It is this unique aspect of Chinese medicine that makes clinical trials in acupuncture a challenge. A study by Birkeflet, Laake, & Vollestad (2010) examined the inter-rater reliability of TCM diagnoses and acupuncture point selections among infertile and previously pregnant women. They found that there was low agreement on diagnoses that identified the individual patterns. The authors concluded that because of poor reliability in the diagnoses, individual treatments received by patients will vary. The same principle holds true for herbal medicine which relies on the treatment principle to prescribe the correct formulation. This represents a limitation to comparing study outcomes since treatments were not made according to differential diagnosis. For this reason it is difficult to say that selected acupuncture points or a specific herbal formula will consistently influence immune cells in a certain way. Time and again, this limitation is tough to overcome since practitioners may differ in opinion regarding diagnosis.

## **Internal and External Validity**

Studies proposing the benefits to Chinese herbal medicine may have significant tension between internal and external validity. In several of the analyzed studies, experimental conditions used in the study do not reflect how the herbs are actually used in practice (Nagamatsu et al. 2007; Ushiroyama et al., 2005). Also, there are significant variations in where the herb was sourced and its preparation. Since traditional herbal prescriptions may be lacking in quality or standardization it is probably difficult to generalize the results from these trials. While internal validity may be high, the research results may not apply to the target population outside the experimental conditions. Also the use of animal subjects in studies on herbs and acupuncture furthers the need for additional studies that evaluate the effectiveness of these treatments on human subjects.

Threats to validity and reliability of this study are minimized for several reasons. The first is that the data were organized into tables simply to report what is described in the analyzed studies, without personal input. Additionally, the majority of articles for this topic are from peer reviewed journals whose acceptance into publications adheres to the standards of practice within the fields of Chinese or allopathic medicine. This increases the reliability and validity of the data collected from each source article. When there was a concern that article content was questionable due to small sampling sizes, observer biases, or an inability to extrapolate from animal subjects to human subjects, it was indicated in Table I.

According to Jiang J.(1997) the treatment principle in TCM for threatened and recurrent miscarriage is to “consolidate the Kidneys, invigorate the Spleen, replenish qi and nourish the blood”. Practitioners of Chinese medicine are taught to cautiously prescribe formulas when

working with pregnant women, especially those with high-risk pregnancies. Herbs that move blood in Chinese medicine may cause potential risk to the fetus if carelessly prescribed. One would think that a blood mover such as Chi Shao which invigorates blood and “dissolves masses” would not be recommended during pregnancy and especially not for one that risks miscarriage. This is because Chi Shao was shown to inhibit anti-inflammatory IL-10 and promotes pro-inflammatory IL-8 (Wang, et al., 2011).

However, Jiang J. (1997) continues with a discussion of the use of blood moving herbs, including Chi Shao during threatened pregnancy. It is important to note that the author acknowledged how single herbs may stimulate the uterus or induce chromosome aberration, but when combined in a formula, blood moving herbs like Chi Shao, can actually help to prevent miscarriage. Therefore, it is rare for a single herbal preparation to be used in practice because infertility patients will often present with multiple patterns. An example of this would be a patient with deficient Blood and excess Damp.

All the herbal treatments in Group C on the effect of herbs on miscarriage consisted either of two or more herbs. There were no herb studies which relied on the properties of one herb in the treatment of miscarriage. With conditions related to pregnancy loss, formulas are likely to be complex. For the treatment of immune-related conditions, one article discussed the properties of a single herb, yi yi ren (Hidaka et al., 1992), and six articles discussed the use of herbal isolates (Tsai, et al., 2011; Li et al., 2011; Wu et al., 2010; Xu et al., 2007; Wang et al., 2011; Zhang et al., 2004). Single herbs or herbal isolate treatments are less likely to be clinically applicable because of individual complexities.

Many of the analyzed studies had small sample sizes which will also contribute to the limitations posed in this project. Additionally, murine models likely do not hold the same

complex emotional patterns as women who experienced trauma from previous pregnancy loss, although one cannot conclude that with certainty. The psycho-social element should be considered in the treatment of recurrent miscarriage or threatened abortion. Mood disorders like depression can enhance pro-inflammatory cytokines (O'Connor, Irwin & Wellisch, 2009). Also, grief from previous miscarriage can change an immunological profile which is why emotional support may benefit recurrent aborters (Liddell, Pattison & Zanderigo, 1991). Because of the differences in emotional intelligence between mice and human subjects, it is difficult to determine if procedures performed on mice are translatable to humans. Additionally, He, Zhao, Wang, and Pan (2010) noted differences in the regulatory mechanisms of human pre-implantation development compared to that in the mice.

There is also the concern of the validity of Chinese medical journals. Studies from China regarding Chinese herbs or formulas have consistently reported positive outcomes in efficacy of treatments in pregnancy-related conditions, but the authenticity and study quality has been questioned (Wu, Li, Bian, & Moher, 2009). Wu et al. (2009) concluded that there is a lack of rigorous clinical standards and design in Chinese studies. Only one of the trials included in this project was randomized. Many of the published studies on Chinese Medicine, especially those in Chinese, lack important details necessary for a valid interpretation of their results. One example of this is that there are frequently studies published on a given property of an herb without any verification that this herb was identified properly.

The primary limitation to this study is the lack of available and synthesized information on the specific topic of how TCM may influence immunological RPL directly in order to support the main hypothesis. Availability is lacking simply due to inaccessibility of journals, because

subscription is lacking, or the majority of relevant texts are in Chinese which runs the risks of bias or information lost in translation. It is also difficult to say whether or not there is enough information available to conclude that Chinese herbs can moderate the immune system to support healthy reproductive outcomes and prevent abortion.

### **Modulation of the Immune System by Stress Reduction with Chinese Medicine and other CAM Practices**

Each year, data reveal around 40% of Americans use complementary and alternative medicine (CAM) and that people who suffer from depression and anxiety (67%) use CAM services significantly more than do their non-anxious and non-depressed counterparts (39%) (White, 2009). While TCM is considered part of CAM, it represents only one aspect.

The concept of the “mind-body” connection is becoming increasingly recognized even in traditional allopathic models, as medicine today appears to be moving more towards integration, (a personal observation). It is recognized that chronic exposure to stress can exacerbate neuropsychiatric disorders such as depression, and symptoms and symptomatic relapses in depressives are attributed to dysfunction of the hypothalamic-pituitary-adrenal-ovarian axis (Mizoguchi, Yuzurugaram, Usgugem, Sasaki, & Tabira 2002). It is also a basic tenet of physiology that stress, depression, and anxiety, if prolonged and intense, can suppress immune system function. Therefore, when either the neuro-endocrine system or immune system suffers, so will the other.

Women are more anxious and have more psychosomatic complaints than their male counterparts (Arranz, Guayerbas, Siboni, & De la Fuente, 2007) which make anxious women

suffering from infertility-related conditions especially susceptible to immune imbalances from stress. The effects of Chinese herbal formulas Xiao Yao San and Saikokaryukotsuboreit-o, (Chai Hu Long Gu Mu Li Tang), were able to moderate glucocorticoid receptor levels in rats under chronic stress (Table I and II). Lughni et al. (2010) recognized that there are acquired inflammatory conditions which have negative impacts on physiologic pregnancy that benefit from glucocorticoid administration. This is because uNK cells express both glucocorticoid and estrogen receptors which is why therapeutic manipulation of these cells is possible (Tang, Alfirevic, Turner, Drury, & Quenby, 2009).

Modulation of immune response by TCM treatment is specific. Multi-herbal formulas are clinically effective partially because of their immunoregulatory properties. This ability to modulate immune function happens in part via macrophage activation and de-activation or through the balance between immune stimulation and anti-inflammatory actions (Novak et al., 2001). Certain herbs and formulas also act as hemostatic agents to stabilize the embryo during threatened miscarriage (Ushiroyama et al., 2006).

Compared with herbal medicine, the data showed that acupuncture as a sole treatment strategy is not as effective for the prevention of miscarriage in women with previous loss. Flaws (n.d.) wrote that when treating threatened miscarriage one should needle points on the top of the head, (such as Du 20 and si shen cong), to raise the qi and support the embryo. He also includes acupuncture points yin tang and ear shen men to calm the patient and support the Kidneys, which according to him, are always indicated in threatened miscarriage. The primary goal of the treatment was to take pressure off the autonomic nervous system. Additionally he lists Sp 1 and LV 1, whose functions are specifically to halt uterine bleeding (Deadman & Al-Khafaii 1994).

Since the Liver has the tendency to stagnate the flow of blood in the body, stuck blood will eventually turn to heat. In Chinese medicine, heat causes agitation which may manifest with disorders that present with reckless bleeding like menorrhagia or any kind of uterine bleeding. The Spleen is responsible for holding the Blood, and Spleen 1 is one of the primary points for strengthening the Spleen's function of holding blood. It is indicated especially for problems with bleeding from the lower half of the body. Deadman & Al-Khafaii (1994) wrote that Spleen 1 is indicated for conditions where excess or deficiency heat enters the blood level to cause reckless bleeding, as is the case with recurrent spontaneous abortion.

According to Zijlstra, van Den Berg-de Lange, Huygen and Klein (2003) acupuncture performed over a long period of time can influence interactions between beta-endorphins and cytokines which could result in an increased formation of anti-inflammatory cytokines like IL-10 and a diminished production of pro-inflammatory cytokines. The autonomic nervous system is responsible for modulating the immune response to stressors hence any treatment that can influence the nervous system, like acupuncture, is a good candidate for immune system regulation.

### **Recommendations for Future Research**

This study found evidence both supporting and contradicting the hypothesis. This is why future studies are so important. One purpose of this project was to investigate whether there are indications to encourage further integration of TCM and CAM therapies in the treatment of recurrent pregnancy loss. The hope is that the information outlined in this synthesis might increase awareness of how Chinese medicine and other complementary therapies can influence the immune system via the psycho-neuro-immune network and how this applies to women who suffer from recurrent pregnancy losses.

In order to uphold the gold standards of evaluating a proposed therapy, several randomized clinically controlled trials are proposed. They should comprise a large number of participants with strict inclusion criteria, such as participants' history should include two or more previous unexplained losses but no additional endocrine imbalance. The treatment interventions should include herbal medicine alone, acupuncture alone, a combination of both or a placebo. The difficulty with placebo or sham acupuncture is that needle insertion can induce a physiologic response in the body (Lundeberg, Lund, Sing & Naslund, 2009). A better solution might be to eliminate the possibility of a placebo effect and give no treatment to the control group. The study design could have four groups. One group would receive herbs, one would receive acupuncture, one would receive both acupuncture and herbs, and the control group would receive no treatment. The purpose of the study would be to test the ability of TCM treatments to change one or more immune variables in groups of pregnant women with a history of RPL. Therefore a placebo might not be necessary in this case (B.Horn, Ph.D, personal communication, 2011).

Levels of specific immune markers should be monitored pre-implantation, every two weeks into the first trimester, and then spaced evenly up until the third trimester, since their numbers vary according to pregnancy stages. While the concentrations of immune cells are significant, live birth rates should also be included as an outcome measure. Live birth rates could then be correlated with concentrations of immune markers. The difficulty with a study like this lies in the ethics of not giving sufficient care to the mother if she begins to show signs of losing the fetus, such as low levels of beta hCG, an abnormal fetal heartbeat, high levels of uterine NK cells, or vaginal bleeding. This issue was raised by Liu and Luo (2009). Participants would have to be informed of the study's objectives and that they may be randomized to the control group.

Uterine NK (uNK) cells were not included in this data analysis since there were no articles found which described the effects of TCM or CAM treatments on these cells. Just like NK cells, the prognostic value of uNK cell levels in predicting subsequent miscarriage is controversial (Choudhury & Knapp, 2000). Uterine NK cells have been detected in the endometrium in the mid-luteal phase and were found to positively correlate with the density of blood vessels in the endometrium of women with unexplained RPL (Quenby, et al., 2009). An increase in blood vessels suggests an increase in potential endometrial blood flow. On the other hand, too much oxygen to the growing fetus can cause the damage to the trophoblast. Oxidative stress from elevated uNK cell levels was found to be responsible for miscarriage (Quenby et al., 2009). Another study concluded that the numbers of uNK cells in the peri-implantation endometrium did not predict pregnancy outcomes, (Tuckerman, Laird, Prakash & Li, 2007). A study that tests the effects of herbs or acupuncture specifically on concentrations of uNK cell levels in women with previous pregnancy loss should be performed. It would be interesting to observe if herbs and acupuncture can lower uNK cell counts in women with elevated levels who are already pregnant.

Ideally, the treatment of infertility and immune-mediated complications of pregnancy should focus on balancing the immune system or reducing the cells at fault. Currently, therapies include immunoglobulin infusions, lymphocyte immunizations and immunomodulatory drugs (Yassin, H et al., 2008). The problem is that these therapies are still experimental and lack evidence from methodically randomized controlled trials and may negatively affect pregnancy outcome (Tang, Alfirevic, & Quenby, 2011). Well-designed studies are necessary to support the therapeutic use of IVIG.

## Conclusion

TCM has been treating miscarriage for thousands of years without a perfect understanding of the mechanism that suppresses or initiates miscarriage. Even today, despite huge advances in medicine, what we know is that we don't know enough. There is incomplete information regarding what exactly is happening inside the uterus during recurrent miscarriage, and about the mechanisms by which herbs and acupuncture can modulate the immune system. Most studies are able to investigate only one or two cytokines as markers for TH1 and TH2 subsets. This represents an oversimplification of the intricacies of the immune system during pregnancy.

Numerous immunological cells are involved in pregnancy loss. What remains unclear is the relative contribution of each. Pregnancy is not a static state and the value of measuring immune markers does not appear to hold great prognostic significance. Hormones and immune cells are in constant flux according to cues from the endocrine system. Their concentrations and activities change at different times in the pregnancy cycle and differ between women. It is this degree of individual variability which may possibly make Chinese medicine's differential diagnosis effective in treating RPL. Based on the reviewed literature, Chinese herbs can promote immunological changes that may decrease the likelihood of miscarriage by mediating pro and anti-inflammatory markers both systemically and locally. Acupuncture may be able to favorably affect the immune system via the central nervous system by increasing in beta endorphins and serotonin, which modulate the immune system (Arranz et al., 2007).

With treatment from Chinese herbs, the body needs to be psychologically ready to absorb the herbal formula. This is accomplished by treating the root cause of RPL which might prevent

the embryo from implanting and which might prevent the patient from fully accepting motherhood. Since a weakness in Kidney function is always implicated in RPL patients, TCM treatments will ultimately focus on strengthening and supporting the Kidney yin, Kidney yang, essence, or all three. To address the psychological aspect of RPL, or the brain and uterus connection, practitioners may include points and herbs for the Heart or Chong meridian. If the egg is not viable then no amount of acupuncture or herbs will help sustain the pregnancy, we can only help nature do what nature does. It is thought that if nature knows best, poor quality embryos will fail to implant. However, in recurrent miscarriage one theory is that this system is suspected to fail (Quenby, Vince, Farquharson, & Aplin, 2002) and embryos that are destined to fail implant only to end up miscarrying. On the other hand RPL might represent the rejection of normal fetuses the maternal immune system. In either instance, if it is the embryo or the maternal immune system, Chinese medicine might be able to offer a little support in the right direction. We can be hopeful that for complicated conditions like recurrent miscarriage, there are several ways to support the patient and that a combination of treatments may help nurture a full-term pregnancy and healthy baby.

## **Appendices**

### **Glossary**

### **Figure I**

### **Figure II**

### **Figure III**

### **Table I**

### **Table II**

### **Table III**

## GLOSSARY

**Allogeneic:** Taken from different individuals of the same species. Being genetically different but belonging to or obtained from the same species as in tissue grafts, hence there is an immunological incompatibility

**Antigen:** A toxin or other foreign substance that induces an immune response in the body, especially the production of antibodies

**ART:** Assisted reproductive technologies. Assisted conception' is the general term that covers the range of medical treatments that are used with the aim of achieving a live birth. The focus of ART is on the use of the more involved medical procedures such as IVF, GIFT and ICSI.

**ASA:** Antisperm antibodies. These are produced in both sexes although women generally possess higher ASA titers than men. They are associated with an immune response that destroys the sperm.

**Cytokine:** A small secreted membrane-bound protein that acts through cell-surface receptors and generally induces changes in gene expression within their target cells. Cytokines produce effects on local cells and work over a short period of time. They also play critical roles in pathogen response and regulation of cellular growth and differentiation.

**Cytotoxicity:** The degree to which an agent possesses a specific destructive action on certain cells or the possession of such action.

**Decidua:** The membrane that lines the uterus and is modified during pregnancy and shed during birth or menstruation.

**Extravillous trophoblast:** The cells that grow out from the placenta and penetrate the decidualized uterus. The extravillous trophoblast is essential to the process by which the placenta attaches to the mother and allows for adequate blood supply to growing fetus.

**Humoral immunity:** The component of the immune response involving the transformation of B cells into plasma cells that produce and secrete antibodies to a specific antigen.

**Immune response:** How the body recognizes and defends itself against bacteria, viruses, and substances that appear foreign and harmful to the body

**Interleukins:** A group of cytokines first found to be expressed by white blood cells (leukocytes).

**MHC:** Major histocompatibility complex which refers to human leukocyte antigen in humans, (HLA). MHC is the term used for all other mammals. MHC is thought to influence pregnancy from gamete development, embryo cleavage, blastocyst and trophoblast formation, implantation through fetal development and survival.

**Miscarriage:** According to the WHO, miscarriage is defined as the loss of a fetus or embryo weighing <500g which would normally be at 20-22 complete weeks gestation.

**Natural Killer Cells:** Small lymphocytes (cells) that destroy other cells without prior sensitization to it. NK cells are the first line of defense against virus-infected cells. These innate lymphocytes have CD3-CD56+ phenotype. These are large granular lymphocytes with natural cytotoxicity. These represent one component of innate immunity. During pregnancy, these cells have the important role of regulating trophoblast invasion.

**Recurrent Miscarriage (RM):** Used synonymously with habitual miscarriage, habitual abortion or recurrent abortion. RM is the loss of three or more consecutive pregnancies before the 24<sup>th</sup> week gestation.

**Recurrent Pregnancy Loss (RPL):** Recurrent pregnancy loss or recurrent spontaneous abortion, (RSA) is the occurrence of 2 or 3 or more consecutive pregnancies that end in miscarriage of the fetus usually before 20 weeks gestation.

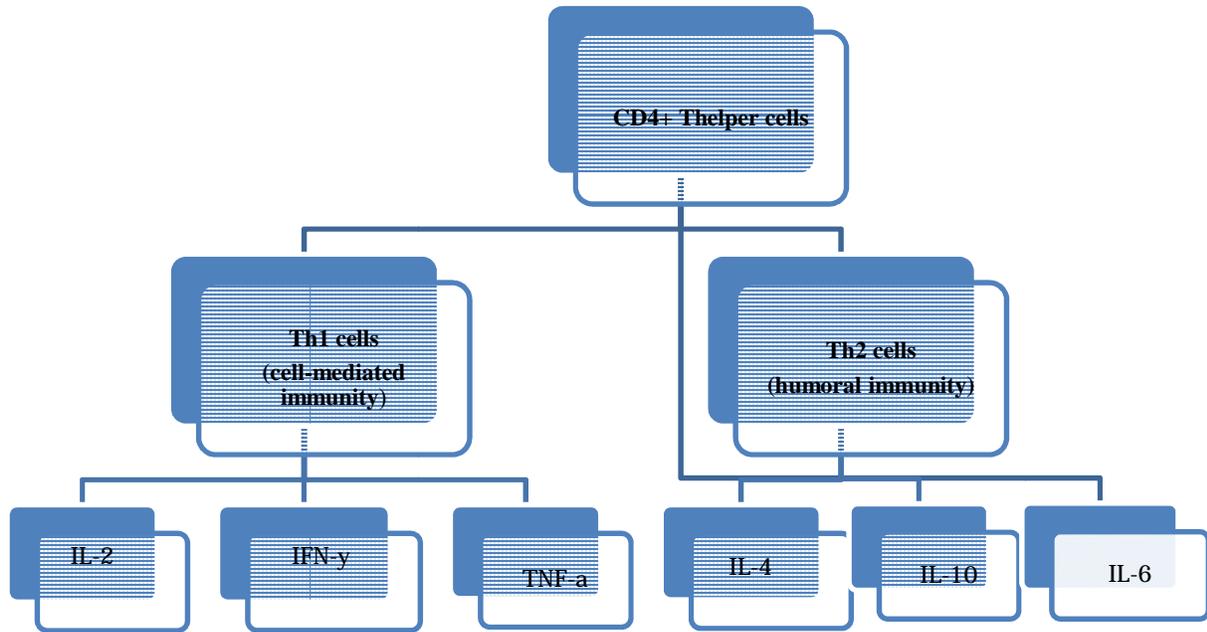
**Retroplacental hematoma:** A pocket of blood clot that accumulates between the basal plate of the placenta and the wall of uterus, causing the placenta to bulge toward the uterine wall into the peritoneal cavity. This in effect may compress the structures and compromise the blood supply to the fetus. This is one of the most common causes of perinatal mortality (85%).

**Spontaneous abortion or miscarriage:** Any pregnancy that is not viable or in which the fetus is born before the 20<sup>th</sup> week of pregnancy. This occurs in at least 15-20% of all recognized pregnancies and usually takes place before the 13<sup>th</sup> week of pregnancy. This is opposed to an induced abortion and is purely accidental

**Trophoblast:** Cells that form the outer layer of the blastocyst to provide nutrients to the embryo. The trophoblast will eventually become the placenta. These are specialized cells of the placenta that aid in embryo implantation and interact with decidualized uterus of the mother

**Tumor Necrosis Factor alpha:** A factor with anti-tumor activity, endogenous and pro-inflammatory

**Figure I: Cell-mediated and humoral immunity and associated cytokine activation**



**Figure II: Cytokine, Source and Principal Activities in the Body**

<b>Cytokine</b>	<b>Source</b>	<b>Principal Activities</b>
IL-1	Activated macrophages	<ul style="list-style-type: none"> <li>• Causes fever, (pyrogen)</li> <li>• Activates endothelial cells</li> <li>• Stimulates T cells</li> </ul>
IL-2	Th1 cells	<ul style="list-style-type: none"> <li>• T cell proliferation and differentiation</li> <li>• Activates macrophages, NK and T cells</li> </ul>
IL-4	Th2 cells	<ul style="list-style-type: none"> <li>• Proliferates and differentiates IgE and B cells</li> </ul>
IL-6	Activated macrophages, T and B cells	<ul style="list-style-type: none"> <li>• Pyrogen</li> </ul>
IL-8	Activated macrophages	<ul style="list-style-type: none"> <li>• Chemotactic factor for many immune system cells</li> </ul>
IL-10	TH2 cells	<ul style="list-style-type: none"> <li>• Anti-inflammatory cytokine</li> <li>• IL-10 expression results in induced killing of intracellular pathogens</li> <li>• Overproduction of IL-10 by T cells was shown to be related to suppressed immunity and increased susceptibility to mycobacteria infection</li> <li>• Suppresses IL-12, TNF-a, and IFN, which results in reduced TH1 cell immunity</li> </ul>
TNF-a	T cells, NK cells, and macrophages	<ul style="list-style-type: none"> <li>• Pyrogen</li> </ul>
IFN-y	Th1 and NK cells	<ul style="list-style-type: none"> <li>• Activates macrophages and NK cells</li> <li>• Inhibits of Th2 activity</li> </ul>

Source: <http://sprojects.mmi.mcgill.ca/immunology/cytokine.htm>

**Figure III: Cytokines Secreted by Th1 and Th2 cells**

Cytokines	Secreted by Th1	Secreted by Th2
IL-2	X	-
IL-3	X	-
IL-4	-	X
IL-5	-	X
IL-6	-	X
IL-10	-	X
IL-13	-	X
TNF-a	X	-
GM-CSF	X	X

**TABLE I**

**Categorical breakdown of each article according to condition(s) treated or study objective, number and type of subjects, treatment intervention, and outcome measure**

Author, Date	Condition/purpose	Subjects/Number	Treatment intervention	Control intervention/#r andomized	Treatment frequency	Outcome measure
Chinese herbs and Immune Function						
Tsai et al., (2011)	Inflammation/Rheumatic pain	Human peripheral blood, n=10	Arcticum Lappa isolate:arctigenin			Effects of AC on T lymphocytes, IL-2, IFN- $\gamma$
Chen et al., (2004)	To test the effects of Yin ZI Huang ingredients on splenocyte proliferation	Mice splenocytes	Yin Zhi Huang herbal injectable			Effects of YZH on expression of T cell activation markers
Li et al., (2011)	Test immunomodulatory activities of medicinal mushroom	Human peripheral blood lymphocytes, murine splenocytes, red blood cells from rats	Purified protein TVC from Fruiting bodies of <i>T. versicolor</i>			Production of TNF- $\alpha$ and nitric oxide in lipopolysaccharide-induced murine macrophages
Wu et al., (2010)	Discover molecular mechanism of immunosuppressive effect of herb ingredient	Rats performed with ectopic peritoneal heart transplantation	Protosappanin A from <i>Caesalpinia sappan</i> L.			T cell proliferation
Xu et al., (2007)	Rheumatoid arthritis Anti-inflammatory effects of anti-malarial agent artemisinin	Human rheumatoid arthritis fibroblast-like synoviocytes, (FLS)	Artemisinin, (artemisinin derivative)			TNF- $\alpha$ induced production of interleukins, (IL-1, IL-6, IL-8)
Wang et al., (2011)	Mycobacterial infection	Primary human blood macrophages	Radix Paeoniae Rubra, (Chishao)			IL-6, IL-8, IL-10 and TNF- $\alpha$
Hidaka et al., (1992)	Verruca vulgaris and verrucae planae	7 healthy volunteers	Coix seeds		6 tablets of coix seeds	Leukocyte counts CD3+CD56+ and

	juveniles, (change in peripheral cytotoxic lymphocytes)				3x/day	CD16+CD57-
Novak et al., (2001)	Atopic dermatitis	Monocytes isolated from peripheral blood of 15 patients with AD and 15 healthy volunteers	Standardized extract from 10 Chinese herbs, Zemaphyte			Morphology and phenotype of developing dendritic cells and their activity towards allogenic T cells
Chinese herbs and stress						
Chen et al., (2005)	Chronic immobilization stress and changes in glucocorticoid receptor in brain regions	Rats	Xiao yao san		180 min daily for 7 or 21 days	Contents of glucocorticoid receptors in hippocampus and immunity reaction
Mizo-guchi et al., (2002)	Chronic stress of water immersion impact on HPA axis, glucocorticoids receptors	Rats	Saikokaryukotsubore it-o		2h/day for 4 weeks	Glucocorticoid receptor level
Chinese herbs and miscarriage or pregnancy rates						
Ma et al., (2009)	Embryonic death rates, IFN- $\gamma$ , IL-10, and progesterone	Bromocriptine treated mice n=100, 5 groups	Baicalin, (component of Huang qin) 3 doses: 10mg/kg body weight, 20mg/kg body weight, 50 mg/kg body weight			Abortion rates, IFN- $\gamma$ , IL-10, and progesterone
Ushiroyama et al., (2006)	Threatened abortion-stabilize early pregnancy	72 women diagnosed with threatened abortion- 1. N=36 hCG, 2. N=36 herbs	Xiong-gui-jiao-ai-tang	hCG		Retroplacental hematoma and bleeding
Zhanget al., (2004)	Recurrent spontaneous	34 women	Baotai granule		10g 2x/day	CD blocking

	abortion					antigen, prolactin, progesterone
Liu et al., (2006)	Abortion rates in IVF and embryo transfer	247 women doing IVF, fresh and frozen	Progesterone +guitai decoction		Guitai started right after transfer and continued until 14 <sup>th</sup> day hCG showed over 25 IU/L	Blood levels of progesterone, bhCG, and estradiol, UTZ
Liu et al., (2005)	Early pregnant women diagnosed with threatened abortion	30 pregnant women, (pi, Shen, or both) 20 control	Zhuyun III		ZYIII 3weeks-how many/day?	IL-2, and IL-10, hCG, progesterone
Zhong et al., (2008)	Restless fetus in early pregnancy	LPS induced abortion mice	Huang Qin and Bai Zhu			IL-10 levels in uterus
Zhang et al., (2004)	Anti-rejection effect in allogenic tolerance	80 rats after allograft transplant	Poria cocos wolf			Survival time of allograft hearts, lymphocyte ratios
Nagamatsu et al., (2007)	Recurrent abortion and intrauterine growth restriction	Cultured human decidual stromal cells from first trimester legal abortion women	Tokishakuyaku-san and Sairei-to			Granulocyte macrophage colony stimulating factor cytokine
Zhong et al., (2002)	Immunological modulation at maternal fetal interface with Chinese herbal medicine	Mice with LPS-induced embryo loss	Radix scutellariae and rhizome atractylodis			NK cells and IL-2
Gui et al., (1997)	Threatened abortion and recurrent spontaneous abortion	Women with TA x=24 Women with RSA x=68	Chinese herbs			Blocking antibody, antiidiotype antibody, cytotoxin antibody, beta-

						hCG, progesterone, estradiol
Sun et al., (1999)	Threatened abortion	40 women with TA	Chinese herbs for reinforcing Kidney, Qi, stop bleeding and antiabortion			Beta-endorphin, GnRH, progesterone
<b>Acupuncture and Immune Function</b>						
Wang et al., (2009)	Surgical trauma and postoperative immune suppression	4 groups of rats 24 each	Electroacupuncture on ST 36 and extra 37 Lanwei	Sham EA		Expression of IL-2, IFN- $\gamma$ , IL-4, IL10, and NK-kB
Joos et al., (2000)	Allergic asthma	38 patients TCM n=20, control n=18 with mild to moderately severe bronchial asthma	TCM: BL13, BL 17, LI4, LU7 with variations and manipulated Control: TE3, TH19, GB8, GB34, and other random points without manipulation	Single blind randomized	12x 30min for 4 weeks	Well-being, eosinophils, CD3+, CD4+, IL-6, IL-10, IL-8, in vitro lymphocyte proliferation
Arranz et al., (2007)	Emotional disturbance/anxiety	34 females suffering from anxiety, 20 controls	19 acupoints 30 min duration			NK cells
Carniero et al., (2010)	Experimental model of asthma	Rats	electroacupuncture			IL-1, IFN- $\gamma$ , IL-4, IL-10, NO
Yamaguchi et al., (2007)	Acupuncture and the immune system	15ml of peripheral blood from 12 healthy volunteers	Acupuncture on liver, spleen, kidney meridians, UB18, UB20, UB 23, ST36			T and B cells and macrophages, and NK cells, CD2+, CD4+, CD8+ CD16+, IL-4, IFN- $\gamma$ ,
<b>Acupuncture and stress</b>						
Song et al., (2009)	Depression and Th1/Th2 imbalance	95 outpatients with major depressive disorder	Electroacupuncture	Fluxetine-n=30 controls		IL-10, TNF- $\alpha$ , IFN- $\gamma$ , IL-4

Acupuncture and implantation and recurrent miscarriage						
Huang et al., (2010)	Connexin gap junction	Rats with blastocyst implantation disorders using mifepristone	Acupuncture			Blastocyst implantation in rats
Stand-ford et al., (2009)	Allergies, recurrent miscarriage	Case study, 33 y.o. female gravid 2, para 0-0-2-0	Acupuncture and allergy elimination desensitization technique		1xmonth for last 5 months of pregnancy. 1x before due date	Progesterone levels, Antithrombin III gene, (ATIII)
CAM therapies and immune function						
Yeh et al., (2006)	Benefits of exercise on physical and psychological health and immune regulation	14 men and 23 women from normal community	Tai chi chuan			White and red blood cells, monocyte, T helper and suppressor cells, IL-10
Waichi et al., (2007)	Breast cancer patients undergoing radiation and chemotherapy	37 women diagnosed stage II and III breast cancer	Yoga postures, breathing and relaxation	Randomized controlled, with supportive therapy	4 yoga sessions per week during radiotherapy with self practice at home	Peripheral blood lymphocytes and NK cell subsets
De et al., (2007)	Job stress and illness	20 Japanese male corporate employees	Recreational music making group drumming		20 controls leisurely reading	NK cell activity
Monit et al., (2004)	Changes in Immunological parameters	29 subjects, Treatment group x=16	Qigong "eight pieces of brocade"	30 min daily practice for one month		Leukocytes, immunoglobulins, and complement
Jones et al., (2001)	Changes in cytokines	19 healthy volunteers	Guolin Qigong			Cytokines, (IL-4, IL-12, IL-6, TNF-a, IFN-y, IL-10, cortisol)







Group 2B: The effects of acupuncture on stress-related variables																					
Song et al., (2009)																				I	D
Group 2C: The effects of acupuncture on miscarriage and implantation																					
Huang et al., (2010)																					I
Standford et al., (2009)																					I
Group 3A: The influence of taiqi, qi gong, yoga, and music-making on immune cells																					
Yeh et al., (2006)																					I
Waichi et al., (2007)	I**																				
De et al., (2007)	I**																				D
Monit et al., (2004)																					NC*
Jones et al., (2001)	I**	I/D		NC	I	w	I	D													D
					7																

Table III

**Total number of increases and decreases of each independent variable (immune cell and other pregnancy marker) after treatment with herbs, acupuncture, or CAM**

	NK cells	TNF-a	IL-2	IL-4	IL-6	IL-8	IL-10	NO	M	NF-kB	IL-8	P	Th1	RH	B	BA	M	CDC4+	CDC3+	CDC8+	GM-CSF	CD57	OS	BE	PR	I	GC	C
Total I	6	3	1	1	1	4	3	1	1	1	1	3	0	0	0	3	0	1	2	0	1	0	0	2	2	1	0	0
Total D	0	2	3	5	3	2	7	1	1	2	0	0	1	1	1	0	2	2	0	2	0	1	1	1	0	0	0	1
Total NC	0	0	0	1	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total M or ID	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Total count	6	6	4	7	5	6	11	2	2	3	2	3	2	1	1	3	2	3	2	2	1	1	1	3	2	1	2	1

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