Does Traditional Chinese Medicine improve semen morphology, motility and count? A pragmatic randomised clinical trial

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abstract

Background

The treatment of male infertility in Chinese medicine is discussed in several cohort studies and case histories recorded and published in Chinese medicine (CM) and biomedicine journals. These papers claim fair to excellent results in improving semen parameters and pregnancy rates. Three clinical trials conducted by Pei et al (2005), Siterman et al (2000) and Siterman et al (1997) also found evidence of a statistically significant increase in semen parameters within the treatment group after acupuncture.

Objective

To compare the effectiveness of acupuncture and Chinese herbal medicine treatment on the semen parameters, count, motility and morphology to no treatment at all.

Design

A pragmatic randomised controlled study.

Setting

Participants received Chinese medicine treatment at a private clinic, the Rozelle Acupuncture and Chinese Medicine Centre, while semen samples were analysed in the laboratory of Sussex Fertility Services.

Patients

Twenty-eight sub fertile men agreed to participate in the study. Of this cohort, 15 participants matched selection criteria and were randomised into the control and treatment groups.

In addition, 13 sub fertile men, who did not meet the selection criteria for the randomised clinical trial, completed a medical history questionnaire and were examined using Chinese medicine diagnosis. In total, 28 sub fertile men were examined and completed the medical history questionnaire.

Measurement

A semen analysis by 'Strict criteria' measured the sperm morphology, motility and count of the treatment and control groups before and after the eight week treatment or control period respectively. The scientist analysing the semen samples was blind to the subjects' status.

Treatment

The acupuncture treatment and Chinese herbal formulas selected were based on Chinese medicine pattern differentiation, individually diagnosed for each participant.

The treatment group (n=8) were provided eight weeks of acupuncture (one treatment per week) and Chinese herbal medicine, while the control group (n=7) received no treatment.

Results

Changes in semen parameters in the treatment and control groups before and after the treatment and control period were compared and analysed using Analysis of Variance (ANOVA), (SPSS).

Statistically significant increases were observed for morphology, $p<0.04$ and motility, $p<0.008$. No statistically significant increase was observed for sperm count $p<0.13$, though a clinically significant improvement in count was observed for the five participants in the treatment group who received Chinese medicine formulas designed to supplement count. The mean increase in count for this group was 220 million sperm (range 63.6 to 826 million).

Fifty-eight percent of participants referred during the study for an ultrasound of the testes were found to have a varicocele, a major factor for male sub fertility.

Limitations

The microscope used to analyse the semen samples was a standard laboratory microscope for this procedure, however it was not able to observe sperm cell nucleus and DNA.

Conclusion

Despite the study having a small number of participants, some significant and encouraging findings towards improvement in sub fertile parameters were observed in the moderately sub fertile participants.

Chapter I: Introduction

1.1 General Background

1.1.1 Sub fertile trends

Several research studies suggest that human sperm counts are declining gradually from one generation to the next in Western societies. Specific estimates claim that average male semen counts have decreased from 113 million per millilitre collected in 1938 to 66 million per millilitre in 1990 and 40 million per millilitre in 2002 (De Jager and Borman, 1995; Jansen, 1999; Taylor, 2003). More seriously, these authors claim that the incidence of sperm abnormalities appears to be increasing, while other research reveals that there is a correlating associated increase in testicular tumors, male babies born with cryptorchidism (when one or both of the testes do not descend) and hypospadias (a developmental abnormality of the positioning of the urethra) (Sinclair, 2002; Taylor, 2003).

One in six couples experience infertility (Taylor, 2003; Cox, 2003) and of this group it is estimated that 40 percent is caused by a male factor, 40 percent is related to a female factor and 20 percent is a combination of both male and female factors (Cox, 2003; McLachlan and de Krester, 2001; Salleh, 2001). Significantly 40 percent of couples that attend an In-vitro fertilisation (IVF) clinic do so as a result of male infertility (Braude and Rowell, 2003a).

1.1.2 Spermatozoa abnormalities

Abnormalities of the head, tail and neck are the most common defects observed and these abnormalities are described as the 'morphology' of the sperm. There are several different types of head abnormalities and each one may severely affect the sperm's ability to fertilise an egg. Abnormalities of the tail may reduce a sperm's ability to swim which is referred to as 'motility'. Morphology and motility along with count were the parameters measured to observe the effectiveness of Chinese Medicine (CM) intervention during the current study.

1.1.3 Sub fertile versus infertile

A man is considered 'infertile' if his ejaculate contains no sperm at all, because he has no potential to conceive naturally. On the other hand a man is considered 'sub fertile' if his ejaculate contains some sperm, but this sperm displays semen characteristics and parameters, (such as count, motility and morphology) *below that which is considered normal.* Any parameter found below normal suggests a reduced capacity of the sperm to fertilise an egg. Therefore the term 'sub fertile' is used when there is a highly reduced conception potential. The current study investigated the treatment of 'sub fertile' men, who have the potential to conceive naturally.

1.1.4 Semen analysis

Semen analysis is the method used to examine a semen sample under a microscope with the purpose of grading specific characteristics including count, motility and morphology, in order to determine whether a man is fertile, sub fertile or infertile. The semen sample is analysed based on the World Health Organisation (WHO) laboratory manual on sperm cervical mucus interaction (World Health Organisation, 1992; World Health Organisation, 1999a). This manual is the basic reference for this investigation.

More recently, research conducted by Kruger and colleagues (Kruger et al., 2004) has led to the introduction of the more rigorous 'Strict Criteria' for analysing semen samples. This method is gradually gaining popularity around the world amongst leading assisted reproduction technology centres (ART) and Andrology laboratories. Strict criteria, was the laboratory standard chosen to analyse semen samples in this study.

1.1.5 Causes of Male sub fertility

The causes of male sub fertility are:

- Idiopathic in more than 60 percent of cases, (thought to be related to either genetic or environmental factors);
- A varicocele in more than 37 percent of cases (varicose vein of the testes);
- Associated with endocrine deficiency in less than one percent;

- Compounded by erectile and ejaculatory disorders in a small unknown percentage (McLachlan, 2004).

1.1.6 Diagnosing the cause of male infertility: the issues and limitations

Some scientists argue that the gradual decline in fertility in modern post industrial societies, has occurred in a relatively short period of time and as a result believe environmental factors are the cause (Sinclair, 2002; Carlson, 1999), others claim genetic and hereditary factors (McLachlan, 2004). No clear consensus exists and determining when specific environmental or genetic factors play a role, remains vague and unspecific leading to a diagnosis of 'unknown cause' in the majority of sub fertile cases.

On the other hand, many scientists emphasise the importance of specifically determining environmental factors in semen abnormalities, in order to identify men at risk of developing further disease related to this exposure (Branigan, 2003; De Jager and Borman, 1999; Sinclair 2002; Townsend, 2004). Similarly, it is also considered important to identify men who have acquired sperm DNA damage as a result of contaminants because this damage may be passed on to offspring through assisted reproduction technology (ART) procedures (Braude and Rowell, 2003b).

1.1.7 Issues related to the limited investigation into the causes of male infertility

In practice, research to identify sub fertile men at risk of developing more serious disease as a result of exposure to environmental contaminants is not pursued in Australia. For example, in Australia research on male infertility has focused on developing a male contraceptive pill and refining technology to by-pass the male factor. The male contraceptive pill research sponsored by pharmaceutical companies has a perceived value in terms of the sale of the developed drug. On the other hand, a lot of money and research over the last thirty years has gone into developing in-vitro fertilisation (IVF) and intra-cytoplasmic sperm injection (ICSI) techniques. IVF is literally fertilisation in a laboratory test tube when natural conception has failed and ICSI introduced to Australia in 1995, provides artificial fertilisation of the female's eggs by injecting the sperm into the egg.

ICSI is extremely good at by-passing male fertilisation problems (Liu and Baker, 2003). It does however attract some strong criticism because of the potential negative health ramifications to offspring as a result of injecting sperm with DNA or chromosomal damage into an egg (Newby, 2003; Hansen et al., 2002; Bartoov et al., 1999). Townsend (2004) argues that while a procedure to by-pass the male factor is appropriate in the short term, if the present decline in sperm count and quality continues at the same rate, within the next 50 years, the average male will be infertile (Townsend, 2004). The cost to the community in providing fertility services as a consequence will be overwhelmingly high, not to mention the potential health costs related to ignoring sub fertile men at risk and not preventing more serious disease as a result of environmental factors.

As a consequence of the above issues and the wider ramifications, there are several good reasons to initiate research into male infertility rather than exclusively focusing on technology to by-pass the male factor. Of relevance, even in acupuncture research, the best studies to date have not focused on investigating the cause of male infertility or supporting natural conception rates, instead the aim of these acupuncture clinical trials was to measure the effectiveness of acupuncture in supporting IVF and ICSI fertilisation rates (Pei et al., 2005; Siterman et al., 1997; Siterman et al., 2000).

1.1.8 Semen analysis: a 'blue print' of a man's health

The main tool used to investigate a male factor is semen analysis. Historically, the main focus and use of semen analysis is in selecting the best procedure and semen sample to support laboratory fertilisation (Windt and Kruger, 2004). A less well known but potentially as important aspect of the semen analysis report is the relationship between semen analysis and a man's general health, including diagnosing, predicting and preventing disease. Apart from fertility evaluation, sperm morphology assessments can be used to observe endocrinology, environmental toxicology (Franken, 2004a), risk of testicular cancer, risk of prostate disease, risk of bowel disease and disease as a result of reduced immunity.

Furthermore, some scientific papers argue that semen analysis may be viewed as the 'fingerprint' or 'blue print' of a man's health (Franken and Kruger, 2004b), because if sperm cells are undergoing DNA fragmentation or show a high incidence of abnormalities, this may be as a result of other health problems or disease in the body, including other cells in the body.

Consequently, all participants in the current study at risk of testicular cancer, or prostate disease were referred to an urologist for further investigation (all men with normal forms below five percent or men with abnormal semen viscosity.) This protocol was adopted based on recommended referral practices from the Australian Centre for Excellence in Male Reproductive Health at Monash University and European specialists (McLachlan and de Krester, 2001; Salleh, 2001), who encourage men at risk to have an ultrasound of the testes every two years.

1.1.9 Infertility in Chinese Medicine

CM, which includes both Chinese herbal medicine and acupuncture, has been used for over 2000 years. CM is a complete system, incorporating the differential diagnosis and treatment of a wide variety of illnesses and disease that afflict human kind.

Infertility was first mentioned in the *Yijing* (Book of Changes), written around the 11th Century BCE (Qiang, 1995). Consequently, further observation and interest continued over the centuries and more recently several clinical trials, cohort studies and case histories published in CM and biomedicine journals claim fair to excellent results in improving semen parameters and pregnancy rates after CM treatment (Baocun, 2001; Becker, 2000; Clavey, 2003; Jiasheng, 1987; Liang and May, 1996; Pei et al., 2005; Siterman et al., 1997; Siterman et al., 2000; Xiangyi, 1997; Xinyun, 1998; Yachun, 1990; Chen et al., 2003; Zhang et al., 2002; Zhiyuan, 1996; Zhiyuan, 1997b; Zhiyuan, 1997a; Zongchang, 1997).

Interestingly, in Chinese Medicine (CM), observing reproductive health as a 'blue print' for the general health of the individual is not a concept without precedent. CM theory

dictates, that if a practitioner wants to understand a woman's health they must look at the associated symptoms related to her menstrual cycle because, it is argued, a women's cycle is a 'blue print' of her health. Up until now we have had no equivalent health blue print for men in Chinese medicine. Therefore access to semen analysis reports and their full interpretation is inevitably appreciated.

1.1.10 The weaknesses of current Chinese medicine research on male infertility and sub fertility

While the claims of these CM studies are encouraging, many of the papers were found to have a poor understanding of the microscopic analysis of semen samples and did not provide evidence of statistical improvement in semen parameters after CM treatment. Furthermore the majority of these studies were either uncontrolled or nonrandomised leading to possible bias and as a result a lesser degree of scientific credibility.

Other noteworthy limitations of these studies include inadequate development of CM diagnosis of male factor sub fertility and insufficient expansion and clarification of treatment strategies, herbal formulas and acupuncture point selection. To compound these particular problems there is significant confusion around the difference between 'sub fertile' and 'infertile' and the different types of semen parameters and abnormalities that were being treated.

At the same time there was incorrect information provided on the processes involved in diagnosing varicocele (a varicose vein of the testes and one of the main causes of male sub fertility). There is limited development of CM formulas to treat varicocele and no semen outcomes or convincing anecdotal evidence to prove CM efficacy in treating varicocele as claimed. As a result, it is considered important to identify this variable (varicocele) and attempt to exclude men from the trial who had male factor sub fertility caused by varicocele.

In summary, these limitations in past studies seem to reflect in general an inadequate development of CM diagnostic and treatment strategies for male sub fertility and

infertility, as well as a neglected use of randomisation, statistical analysis and semen analysis to measure outcomes after CM intervention by the researchers.

1.2 Significance of the study

1.2.1 The strengths of the Chinese medicine model: supporting the current study

On the positive side, it is pertinent to note that CM has a history of emphasising a methodological process of investigation and problem solving that was found to complement scientific models of research in this study. CM anthropologists suggest that CM is constantly in a process of evolution and development with its core purpose being one of investigation and problem solving (Scheid, 2002a) by taking into account all details, associations and variables.

As a result it was considered imperative in the current study to ascertain the cause of the sub fertility from a CM perspective by constant re-evaluation of current CM diagnostic and treatment strategies. An emphasis was placed on updating and amending current CM strategies based on the clinical evidence presented. From structured detailed observation a tailored treatment program was delivered to the participants. This 'pragmatic' treatment approach was considered more clinically valid than offering one formula to suit all participants because of the many unknown variables involved in male factor sub fertility. The lack of knowledge into the specific causes of male sub fertility strongly influenced the design of the current study. For example, a clinical trial investigating female infertility would identify known categories such as polycystic ovary syndrome (PCOS), endometriosis, obstructed fallopian tubes, early menopause, anovulatory cycles, fibroids and mennorhagia. One formula, drug or intervention would not suit each one of these groups. Measuring the effectiveness of any intervention would focus on each category separately.

Given the purported claims that CM can treat male sub fertility it was decided to conduct a randomised controlled pilot study on the effect of CM intervention on moderately sub fertile men. It was also considered important that the issues related to diagnosis, condition specific variables, treatment strategies, statistical analysis, randomisation,

measurement of outcomes through semen analysis, referral practices and whether or not a semen analysis can be seen as a 'blue print' of a man's health, needed to be investigated further. As a pilot study in the form of a randomised clinical trial (RCT), this research project chose to place an emphasis on a broad scope of investigation, such as monitoring the effectiveness of CM treatment through RCT and observing the medical history profile of participants in order to support an understanding of causative factors.

In summary, if the implications of semen analysis are far broader than the current application in the IVF setting, laboratories independent of the IVF centre may play a key role in further investigation of the semen sample with a view to predicting and preventing disease. Examining CM's effectiveness in treating male sub fertility in light of the broader implications and CM's potential effectiveness in treating these associated conditions was also considered of interest.

An RCT is seen as the gold standard of research (Lewith et al., 2002) and was chosen in this pilot study to assist in eliminating bias. The scientist measuring outcomes, James Leong was 'blind' to the participants' selection and this also contributed to reducing bias.

1.3 Aim of the study

The aim of this study was to:

- Determine whether Acupuncture and Chinese herbal medicine treatment improves sub fertile semen parameters.
- Document and analyse the general health profile of the sub fertile men according to CM disease categories through a thorough medical history questionnaire.

1.4 Format of Thesis

Chapter II Background

Chapter Two outlines important definitions and background information crucial to understanding the design of the study and the selection process. This chapter also

introduces the CM theories on the diagnosis and treatment of male sub fertility and the adaptation of these theories to the current study.

Chapter III Literature Review

Chapter Three reviews CM research on male sub fertility. Clinical trials and cohort studies are reviewed for their strengths and weaknesses in the context of the present research.

Chapter IV Method

Chapter Four explains the design and implementation of the study by detailing the exclusion criteria, recruitment, treatment procedure, the method used to measure outcomes and procedures used to analyse statistics.

Chapter V Results

Chapter Five reports the main sub fertile parameters measured before and after treatment. This chapter also documents the medical history and common and significant health findings highlighted in the questionnaire completed by all participants. Also reported are the answers to the feedback form completed by participants in the RCT.

Chapter VI Discussion

Chapter Six discusses the results of the RCT and the medical history questionnaire and raises implications and recommendations for CM clinical practice and further research.

Chapter VII Conclusion

Chapter Seven summarises the findings and comments on future research directions.

Appendices

References

Chapter II: Background information that influenced the design of this study

2.1 Clarification of important definitions

2.1.1 Varicocele

A varicocele is thought to cause an increase in blood flow to the testes, creating a rise in temperature that increases the number of sperm abnormalities and the development of a more serious level of sub fertility. It is estimated that over 37 percent of sub fertile men have a varicocele (McLachlan, 2004) however this figure may not be accurate because it is based on medical opinion rather than the results of a large scale observational study.

Varicocele can often be seen by visual observation and palpation of the testes; however a visual examination is not conclusive. A thorough diagnosis to detect an internal or '*sub clinical*' varicocele requires an ultrasound of the testes evaluated by an urologist that specialises in male infertility (Evers and Collins, 2003; Jarow et al., 1996). If a positive diagnosis is determined the urologist may suggest surgery such as ligation, or a less invasive procedure called embolisation, to reduce the impact of the varicocele.

A recent systematic review on the benefits of varicocelectomy (Evers and Collins, 2003), concludes that there is no convincing evidence after assessing relevant RCT's that varicocelectomy significantly improved semen parameters. Despite this recent report, varicocelectomy continues in Sydney and these findings increase the importance in investigating the effectiveness of CM treatment on this group.

The question of the actual incidence of varicocele among participants and the required diagnosis using ultrasound to more accurately identify a potential varicocele was an important consideration during the current study. Resources, such as ultrasound screening for participants were not available and this limitation influenced participant selection to eliminate men who potentially had varicocele. Men with normal semen forms below four percent were considered a high risk for varicocele.

At the same time it was considered important to ascertain, what percentage of this group (normal forms below four percent) did actually suffer from varicocele. As a result, a system of voluntary deference for ultrasound was suggested to men at risk, as this information might be useful to the participant and the study.

2.1.2 The semen analysis form.

A semen analysis form is used to document observations of each semen sample. The semen analysis form (Appendix 2) is based on strict criteria and WHO specifications used in the laboratory of Sussex Fertility Services. The same outcome measurements were used in this pilot study. Below is an explanation of some of the most important parameters.

Volume: A healthy ejaculate measures between two to six millilitres (ml).

Sperm Density ($x10^6$): Sperm density refers to the sperm count per ml collected. Sperm are usually counted by the million.

Total Sperm Count ($x10^6$): The total count is calculated by multiplying the volume by the count per ml.

Live Sperm morphology (%): Morphology refers to the shape of the sperm and whether it is normal or abnormal. The results will indicate the percentage of 'normal forms' found in the sample.

Vitality %: Refers to the percentage of live sperm found in the ejaculate.

Abnormal sperm morphology: This area of the analysis form divides the abnormal sperm percentage into the head, neck and tail.

Seminal Sperm antibodies (Immunobead test): Sperm coated with antibodies may die or adhere to other sperm leading to compromised motility and fertilisation potential. In this instance the immune system identifies the sperm as being a foreign body and as such coats them with antibodies. Testing positive for sperm antibodies is problematic when the total number is greater than 50 percent.

2.1.3 Normal versus sub fertile semen parameters

Scientific studies show that semen ranges below certain levels predispose a man to being sub fertile. These sub fertile ranges lie well below the average, however the accepted 'average' is significantly and gradually declining in populations in the West (De Jager and Borman, 1995; Jansen, 1999; Taylor, 2003). The parameters that establish a man in the sub fertile category include:

- Volume less than two ml per ejaculate.
- Sperm density less than 20 million.
- Total sperm count less than 40 million.
- Normal forms less than 30 percent.
- Sperm motility less than 30 percent.
- Sperm antibodies greater than 50 percent.

(Jansen, 1999; World Health Organisation, 1992; World Health Organisation, 1999a).

2.1.4 Sub fertile parameters that influenced the selection criteria

Research suggests that morphology or the number of normal forms in a semen sample has the greatest influence on fertilisation potential (Carlson, 1999). More specifically, in 1996 Coetzee et al (1998) conducted a literature review on studies published between 1978 and 1996 (Coetzee et al., 1998; Windt and Kruger, 2004) and found that 92 percent of papers claimed that in particular, head defects and normal forms below 15 percent were a positive predictor of fertilisation potential in vitro.

Therefore, many studies investigating the impact of morphology on fertilisation rates conclude that a lower percentage of normal forms more accurately predict sub fertility

than is suggested by the WHO guidelines. WHO guidelines suggest normal forms below 30 percent while Coetzee and his colleagues found normal forms below 15 percent as a positive predictor.

Kruger (1986) found that based on morphology sub fertile men could be divided into three distinct groups to predict fertilisation potential during in vitro. These groups were, the P-pattern group, or poor prognosis group (zero to four percent normal forms), the G-pattern group, or good prognosis group (five to fourteen percent normal forms) and the N-pattern group or normal group (normal forms greater than fourteen percent). Kruger et al went on to suggest that the P-pattern group or those men with normal forms below four percent experience the lowest fertilisation and pregnancy rates in IVF laboratories 1986 (Kruger, 1986).

All the studies reviewed, researching fertilisation potential, focused on the IVF laboratory work, rather than natural conception potential, even so, the profiles offered by Kruger et al, provide the best guidelines available to divide men into sub fertile categories. These profiles were used as a guideline to the selection criteria in the present study. Participants from the G-pattern group or the good prognosis group (five to fifteen percent normal forms) were selected to be randomised, profiled and treated with acupuncture and Chinese herbal medicine. Subjects in the P-pattern group or poor prognosis group (zero to four percent normal forms) were the group that were observed and profiled only. The N-pattern group or the normal group, (16-30 percent normal forms) were excluded from the study.

1.1.5 The major issues of semen analysis

The limitations of semen analysis are important to consider when measuring outcomes, or the results after an intervention such as Chinese herbal medicine and acupuncture on sub fertile men, because while semen analysis is the obvious choice in measuring outcomes, the limitations of the semen analysis report may lead to the failure of the analysis to detect subtle but clinically significant improvements after the intervention.

For example, this might especially apply in the case of very sub fertile men (or the P-pattern group) with a high risk of DNA fragmentation that might not be identified using the standard semen analysis microscope. For example the microscope used to examine semen samples in an IVF clinic or Andrology laboratory magnifies the sperm by 200-400 times their normal size (World Health Organisation, 1999b) and does not allow the scientist to observe the cell nucleus contained in the head of the sperm. Therefore evaluation of sperm morphology does not normally include observing genetic, chromosomal or DNA damage. As a result, improvements in these areas after an intervention are also not detected.

On the other hand, because of the low magnification of the semen sample, some sperm can appear normal but still have undetected chromosomal and DNA abnormalities (Bernardini, 1997; Calogero, 2001; Kunathikom and Rattanachaiyanont, 2002; Lee et al., 1996; Ryu, 2001). This is more likely to be the case in semen samples with high levels of abnormal sperm.

As a result, many researchers recommend the use of the more thorough diagnostic techniques, such as Transmission electron microscopy (TEM) and the Acrosomal Index test (AI) in conjunction with strict criteria semen analysis (Pei et al., 2005; Siterman et al., 1997; Siterman et al., 2000; Zhang et al., 2002). These diagnostic tools more accurately predict sub fertility and fertilisation rates than morphology alone.

Notably, these investigative techniques are not routinely used in the laboratory in conjunction with standard semen analysis. This is thought to be significant when measuring outcomes after intervention such as Chinese medicine, especially in the very sub fertile group (P-pattern group, or zero to four percent normal forms). As a result significant changes after CM intervention may not be observed due to the limitations of the standard semen analysis procedure.

Several papers also criticise the process of analysing semen samples (Franken, 2004b; Jequier and Ukombe, 1983; Menkveld and al., 1997; Morgenthaler et al., 1995; Mortimer

et al., 1986; Neuwinger et al., 1990). Lack of standardisation and quality control of this process leads to potential errors and misinformation when diagnosing sub fertile men. These papers suggest that even specialist IVF clinical laboratories have an unacceptably high error ratio when analysing semen samples, resulting in false positive reports.

Consequently it is recommended that all laboratories analysing semen samples adopt the protocols of 'Strict Criteria', including internal auditing, continuing education, training and testing for consistency and accuracy. Laboratories that fall short of this requirement are not considered reliable and it is suggested that sperm DNA and Acrosome index tests used in conjunction with strict criteria semen analysis is the most accurate and therefore the fairest method of assessing outcomes after intervention in clinical trials investigating male sub fertility.

Significantly access to sperm DNA and Acrosome index tests was not available to this current study and this notably influenced the selection criteria to exclude the P-pattern group or participants with zero to four percent normal forms in the main randomised group. Importantly, this sub fertile group with normal forms below four percent were still observed and profiled to support the collection of data and statistics.

2.2 Chinese medicine and male infertility

2.2.1 The Meridians

CM theories claim that the meridian system is a complex network of channels that travel both along the surface of the skin and internally. The meridians form channel like pathways that connect and interact with all major organs and systems in the body. An in-depth understanding of this system is the foundation of Chinese medicine. When studied, and skillfully used, Chinese medicine may regulate and normalise the activity of every other system in the body, including for example the hormonal, immune, digestive, nervous, muscular skeletal, cardiovascular and reproductive systems.

Both acupuncture and Chinese herbal medicine work on the meridian system by stimulating the body to potentially maximise the healing response. For example,

acupuncture and Chinese herbal medicine cannot make a person grow a new finger because it is not normally possible. However whatever is possible, such as liver detoxification of environmental poisons, or the immune system response to a virus or bacterial infection CM therapy can enhance (Bensky & Gamble, 1986; Rogers, 1981).

2.2.2 General investigation and diagnosis in CM

CM has a unique and practical method of diagnosis that is investigative and analytical. When all the possible factors contributing to a disease are identified by a thorough CM diagnosis, appropriate acupuncture and Chinese herbal formulas are prescribed. Rather than just focusing on treating the disease, all associated diseases and symptoms are treated with a view to re-establish equilibrium and therefore health. This approach also requires the practitioner specialising in male sub fertility to address all associated health issues such as infections, or digestive and nervous system disorders. This process of treating the whole body is a principle of holistic medicine and CM theory is a forerunner of this approach.

In support of this philosophy, CM has developed very complex and insightful diagnostic and treatment protocols on a broad variety of diseases. This is reflected in the vast volumes of writings on acupuncture and Chinese medicine herbal formulas and strategies based on centuries of experience and observation. This is one of the great strengths of the CM system that all practitioners rely upon. However, at the same time, this system also encourages constant re-evaluation of known protocols and a CM's physician's skill is measured not only by their understanding of the great classics and their ability to accurately apply thousands of formulas, but also in the versatility and the agility of their diagnosis.

Schied (2002) explains this well when he states,

> "While standard additions and subtractions (*jia jian*) exist for many formulas, these do not dictate practice. Rather, they are models from which the virtuosity of a physician develops. This virtuosity is referred to by Chinese

> doctors themselves with terms such as ling and *linghuo* which translates as ‘agile’, ‘quick’, ‘nimble’, ‘flexible’, ‘elastic’, ‘adaptable’. A physicians *linghuo* characteristics are expressed emblematically in the composition of his or her prescription: in its suitability to present context, its adaptable resources, its clever composition and therefore in its spirit like efficaciousness” (Scheid, 2002b).

2.2.3 CM diagnosis and investigation of male sub fertility

While some scientists argue male sub fertility is predominantly caused by environmental contaminants and other medical experts claim the mostly idiopathic causes are as a result of hereditary and genetic factors (Carlson, 1999; McLachlan, 2004), CM theories emphasise openness in observation while taking into account all variables. As a result CM identifies several major causes of male sub fertility as well as environmental, genetic and hereditary influences.

This is achieved by the use of an extensive checklist for every patient examined. This check list investigates the potential affects of the:

- Environment
- Diet
- Parasites, viral or bacterial infection
- Emotional distress
- Hereditary
- Trauma
- Climate

In identifying several major factors, it may be argued that CM’s method of investigating all variables is very practical when analysing idiopathic causes. Subtle symptoms that are overlooked or underestimated by medical diagnosis may be recognised as important in

CM. Useful links and associations may be made during diagnosis. Joining the dots may help to provide answers while at the same time complementing and satisfying scientific research requirements.

In order to undertake a comprehensive diagnosis of these factors a thorough structured medical history questionnaire is taken. This is then complemented with the information gained by using three diagnostic tools that are unique to Chinese medicine. These tools include:

- Pulse diagnosis
- Tongue diagnosis
- Palpation of meridians

These tools do not restrict a CM practitioner's use of other available forms of investigation to validate and complement findings. Sub fertile men are often referred for further testing such as:

- Semen analysis
- Ultrasound of the testes
- Blood tests
- Hair analysis

Therefore, the CM practitioner specifically looks to assess the cause of male sub fertility through the use of its diagnostic methodology and a detailed understanding of the semen analysis report. Combining these two tools may prove to be a useful way to more fully understand the unknown causes of male sub fertility and identify men at risk of more serious disease, for example, as caused by significant exposure to environmental contaminants. Once a thorough investigation is completed a treatment protocol is established and a specific and individualised treatment program is designed.

2.2.4 CM diagnostic and treatment categories in male sub fertility

Four main syndrome categories are historically listed as relevant to male sub fertility. These categories are:

Liver Qi Stagnation
Damp Heat
Kidney Yin Vacuity
Kidney Yang Vacuity
(Becker, 2000; Chen and Wen, 1996; Clavey, 2003; Crimmel et al., 2001; Jiasheng, 1987; Minghua, 1993; Xiangyi, 1997; Xinyun, 1998; Yachun, 1990; Yang, 2001; Zhiyuan, 1997b; Zhiyuan, 1996; Zhiyuan, 1997a).

For the comprehensive coverage of possible syndromes associated with male sub fertility, another five categories were added to the above. These are:

Toxic Liver (as a result of environmental contaminants)
Spleen Qi Vacuity
Parasites
Blood Stasis
Wei Qi Vacuity

The 'toxic liver' category is a new category used to help classify the impact of significant environmental contaminants on sub fertile men. Other single herbs related to liver yin vacuity, yin vacuity leading to wind, cold damp spleen, blood vacuity, liver yang rising and heart blood vacuity are also considered as possible important additions depending on the symptoms of the individual.

Chapter III: Literature review

3.1 Chinese Medicine (CM) research on male sub fertility

The effectiveness of CM treatment of male infertility and sub fertility is discussed in several studies and case histories recorded and published in journals of both Chinese medicine and biomedicine. The intention of this review is to analyse the studies (i) that conducted a clinical trial, (ii) were a cohort using Chinese herbal medicine, (iii) were a cohort using acupuncture (iv) and were a cohort using acupuncture, Chinese herbal medicine and point injection therapy.

The studies reviewed were located by searching through a collection of Chinese medicine journals in the library of the University of Technology, Sydney and by purchasing a CD of The Journal of Chinese Medicine containing back issues of over twenty years. Electronic data bases were also searched. These included Science direct, Medline, Ovid, AMED, Health Star and CINAHL. In total, eleven studies were found that used either acupuncture, Chinese herbal medicine or a combination of acupuncture and Chinese herbal medicine to treat sub fertile men. Three clinical trials used acupuncture to treat sub fertile men, five cohort studies used Chinese herbal medicine to treat sub fertile men, two cohort studies used acupuncture and one cohort study used a combination of acupuncture, Chinese herbal medicine and point injection therapy.

3.1.1 Clinical trials using acupuncture

In three recent studies acupuncture was used in conjunction with Assisted Reproductive Technology (ART) to improve fertilisation rates for men who had abnormally low sperm counts which resulted in difficulty fertilising eggs using Intra-cytoplasmic sperm injection (ICSI). The very sub fertile men required the support of ART and were matched based on poor fertilisation rates while using ICSI. Siterman et al (2000) conducted a prospective controlled study with 40 men divided into the treatment and control groups. The participants in the treatment group underwent ten acupuncture treatments over five weeks and their semen samples were measured before and after intervention. The control

group, a wait list control was carefully matched to the treatment group however the men were not randomly allocated. The control groups' semen samples were tested before and after the control period. They did not receive any acupuncture treatment. The semen samples were collected after 17 days abstinence. This was an unusually long abstinence period, as semen abnormalities may occur after eight days abstinence and a sample is usually requested between three and seven days abstinence. Semen samples were studied using a light microscope called special micro electron scanning microscope (mSEM) and this provided a very detailed analysis. The research team had access to some excellent equipment and powerful microscopes that were capable of measuring very subtle changes in semen samples after intervention. The acupuncture treatment provided in the study was clearly explained. The team reported a clinically significant and statistically significant outcome ($p<0.02$). Two pregnancies were achieved by ICSI after the treatment period. There was no clinical or statistical change observed in the control group's semen samples. The results also suggested that the sub fertile men who had testicular infection, responded particularly well to acupuncture treatment (Siterman et al., 2000).

Earlier, Siterman et al (1997) conducted a prospective controlled study matching 32 sub fertile men, based on varicocele, history of prostatitis; low sperm count; poor motility; and poor morphology. The treatment group received acupuncture twice a week over five weeks and semen samples were examined before and after the treatment period. The control group received no acupuncture treatment and their semen samples were tested twice for comparison within the same time frame as the treatment group. The laboratory scientists were 'blind' to the subject's status. Promising results were shown with significant improvements being measured in three basic semen parameters, sperm viability ($p<0.02$); total number of motile spermatozoa ($p<0.02$); and the mean total functional sperm fraction (TFSF) ($p<0.004$); which includes sperm count, motility and morphology. The team measured the semen samples based on a laboratory protocol called 'the fertilization index' and had access to a wide diversity of investigative facilities, such as blood tests, ultrasounds and assessment of sperm DNA and AI indexes through the use of an electron microscope. Interestingly the authors found that nearly half the participants had varicocele (46%) and another 50 percent had prostatitis. Unfortunately

the participants were not randomised, making the study's results difficult to interpret (Siterman et al., 1997).

More recently, Pei et al (2005) conducted a study on the effect of acupuncture on semen parameters as assessed by a transmission electron microscope (TEM). Due to the severe sub fertile nature of the semen samples, ultrastuctural defects such as acrosome position and shape, nuclear shape, axonemal pattern and shape and accessory fibers of sperm organelles were examined on the 40 participants' before and after intervention. Twenty eight participants were allocated to the treatment group and 12 participants to the control group, therefore based on size, the groups were not well matched. Participants were not randomised and were aware of their selection and status. In addition there was no placebo controlled group. The treatment group received two acupuncture treatments a week over five weeks. TEM was conducted on semen samples of both groups before and after the five week treatment or control period. The selection criteria excluded participants with infectious disease or immunologic associated disease and while this resulted in high internal validity it also resulted in poor external validity because the participants are not representative of the general sub fertile population. Men who were infertile (azoospermic) were also excluded from the study. The scientist evaluating the semen samples was blind to participant selection. The results showed a statistically significant improvement in count ($p<0.02$); acrosome positioning ($p<0.1$); acrosome shape ($p<0.001$); nucleus shape ($p<0.001$); normal axoneme pattern and shape ($p<0.005$ and $p<0.2$ respectively); and accessory fibers ($p<0.005$) in the treatment group. Whether or not a clinically significant increase in semen parameters was observed after intervention was not reported. In summary, this study supported the use of acupuncture in sub fertile men who required the assistance of ART procedures such as IVF or ICSI (Pei et al., 2005).

The three clinical trials reviewed above used electron microscopy to measure detailed improvements in semen parameters, such as observing sperm acrosome and cell nucleus. This is a more detailed and fairer method of assessing an intervention however access to this type of microscope is limited to specialist university laboratories. While encouraging, none of the clinical trials were reported to be randomised and none of the studies

measured the effectiveness of Chinese medicine treatment on semen parameters to support natural conception in moderately sub fertile men. Therefore the effectiveness of Chinese medicine in treating this group remains unknown.

3.1.2 Cohort studies using Chinese herbal medicine

Yachun et al (1990) conducted a study on 82 patients who were prescribed a CM herbal formula called *Ju Jing Tang* powder for nine months and claimed a total effectiveness rate of 85.4 percent on the parameters of sperm count and motility ($p<0.01$). The study was non-randomised and there was no control group. Base line measures for count, motility and morphology were given before intervention and then after intervention suggesting that the study was designed well. The CM formula prescribed to participants was based on the main symptoms of the individual. The study design is a good example of a pragmatic approach to treating male sub fertility (Yachun, 1990).

Ishikawa et al (1996) conducted a cohort study on 37 men diagnosed with varicocele. They were prescribed *Gui Zhi Fuling* W*an* for three months. Participants' varicocele was graded before and after treatment and the results suggest this formula reduced the size of the varicocele, but only mild, grade one varicocele. The study was non-randomised and did not have a control group. Of importance, morphology was not investigated, which is the main parameter that varicocele impacts upon. Therefore it was not clear whether or not the participants were sub fertile based on morphology and whether CM herbal treatment improved morphology. While the results claimed to show a statistical improvement in count ($p<0.05$), the improvement was not clinically significant (a mean increase of 15 million only). Improvement in motility was both statistically and clinically significant ($p<0.01$) (Ishikawa et al., 1996).

Chen et al (1996) conducted a study on 202 sub fertile male participants prescribed *Sheng Jing Tang*, for 60 days. Despite the large numbers, there was no control group and the participants were diagnosed as infertile when they were sub fertile. Morphology was not measured before or after treatment and all participants was prescribed the same formula despite 77 percent being diagnosed with kidney yang vacuity and 33 percent being

diagnosed with kidney yin vacuity. No attempt was made to identify men with varicocele. The study reported a 78 percent pregnancy rate in 148 couples who were available for follow up. Improvements in sperm density, motility, levels of follicle stimulating hormone, luteinising hormone and testosterone were also claimed. (Chen and Wen, 1996).

Baocun et al (2001) conducted a study on 87 sub fertile men. The men were all prescribed one to three months of CM herbal treatment based on the formula *Sheng Jing Zhong Zi Tang*. Variations of the formula were provided based on CM patterns of diagnosis and the dominance of either kidney yin or yang vacuity. For example 32 participants were diagnosed with kidney yin vacuity, 28 participants with kidney yang vacuity and 27 participants with kidney yin and yang vacuity. Count, motility, morphology and agglutination were examined before intervention, however only count and motility scores were recorded and used to show outcomes after treatment. The results suggest an overall 95 percent total effectiveness rate and a p value of $p<0.05$. The group was non-randomised and the control group did not match the treatment group in base line semen parameters because they were not sub fertile. There were 87 men in the treatment group and 30 participants in the control group and therefore based on numbers, the groups were not well matched. While a CM diagnosis was undertaken to divide the participants into CM diagnostic groups, it was not comprehensive and no identification of varicocele was undertaken. The mean age of the men was 29.6 years. This is a fairly young age group and while the mean age of their partners was not given, this was significant because achieving a pregnancy was calculated as part of the effectiveness rate (Baocun, 2001).

Chen et al (2003) conducted a study on 36 sub fertile patients with chronic prostatitis based on a CM diagnosis of blood stasis. The participants were prescribed 60 days of the Chinese medicine formula *Shao Fu Zhu Yu Tang* to measure the effectiveness of improving sperm count and motility. Semen analysis results were documented before and after the 60 day treatment period. Motility, count and acrosin activity were assessed. The results revealed a significant improvement in sperm motility after treatment ($p<0.01$) and acrosin activity ($p<0.01$). Despite being a relatively recent study, there was no analysis of

morphology before and after treatment and this was a non randomised study. The results were achieved by comparing the base line measurements of the semen parameters of the treatment group to their post treatment results. There was no comparison to a control group. The herbal treatment was provided in a capsule, therefore a placebo treatment was possible though for some reason not incorporated into the study (Chen et al., 2003).

3.1.3 Cohort studies using acupuncture

Jiasheng (1987) conducted a study on 248 sub fertile men, providing acupuncture treatment over a 20 day period. The participants were given treatment based on a diagnosis of kidney yang vacuity, kidney yin vacuity, damp heat or liver qi stagnation. The results reported improvements in 74 percent of participants. There was no randomisation and no control group. Base line measurements for semen parameters were not given. Therefore there remained no concise evidence of improvement despite the claims (Jiasheng, 1987).

Minghua (1993) conducted a study on 39 men, providing the same combination of acupuncture points selected for the treatment given several times per week over a 90 day period. The study aimed at improving sperm count and motility and found an overall 'cure' rate of 54 percent in semen parameters measured. The main 'cure', was the participants' partner achieving a pregnancy. There was no control group and the selection criteria did not exclude men who were fertile, therefore there was no true initial diagnosis of sub fertility. An assessment of morphology was not given (Minghua, 1993).

Zhiyuan (1997) conducted a study on 54 sub fertile men providing 30 acupuncture and moxabustion treatments and reported that 100 percent of the participants had improvements in abnormal semen parameters with a 68 percent pregnancy rate after the study was completed. The participants were non-randomised and there was no control group. The study provided poor evidence of baseline semen parameters before and after intervention (Zhiyuan, 1997a).

3.1.4 Cohort study using acupuncture, Chinese herbal medicine and point injection therapy

Zongchang (1997) conducted a study on 174 men diagnosed with several complaints that appear to reflect scientific investigation influenced by cultural bias. The complaints included azoospermia (no sperm in the ejaculate), sub fertility complicated by prostatitis, testicular inflammation and masturbation. One hundred and fifty men were randomised into five groups. The men were not matched by a placebo control group. One group of participants received acupuncture treatment, point injection of pilose antler and Chinese herbal medicine. The second group of participants received acupuncture treatment and point injection of pilose antler. The third group of participants received Chinese herbal medicine and acupuncture treatment. The fourth group of participants received acupuncture treatment only. The fifth group of participants received Chinese herbal medicine and injection of pilose antler. The remaining 47 participants received acupuncture, Chinese herbal medicine and injection of pilose antler. The men who suffered prostatitis were also prescribed antibiotics. Treatment was offered over a two month period. The CM medicine formula used was standardised for all participants. There did not appear to be any attempt to diagnose the men using CM, or identify varicocele. Count and motility were measured before and after intervention. Morphology was not measured. This was an interesting study attempting to measure a number of treatments against each other. The results suggest a 92.9% effectiveness rate in the group that received acupuncture, Chinese herbal medicine and point injection therapy (Zongchang, 1997).

A general assessment of the studies covered suggests that while the claims appear to be encouraging and certainly justify further research, the majority of these papers were in-adequate in a number of important areas. Significantly it appears that these studies were either uncontrolled or nonrandomised leading to possible bias and as a result a lesser degree of scientific credibility. While a percentage of success was stated, the majority of papers failed to provide evidence of improvement in semen parameters after CM treatment by measuring semen parameters before and after intervention. In particular morphology was greatly ignored.

Chapter IV: Method

4.1 Participants

Twenty-eight sub fertile men completed consent forms and 15 participants met inclusion criteria. Thirteen men did not match the inclusion criteria for the randomised part of the study but were eligible to participate in the medical history questionnaire. The participant allocation process is shown in Figure 4.1

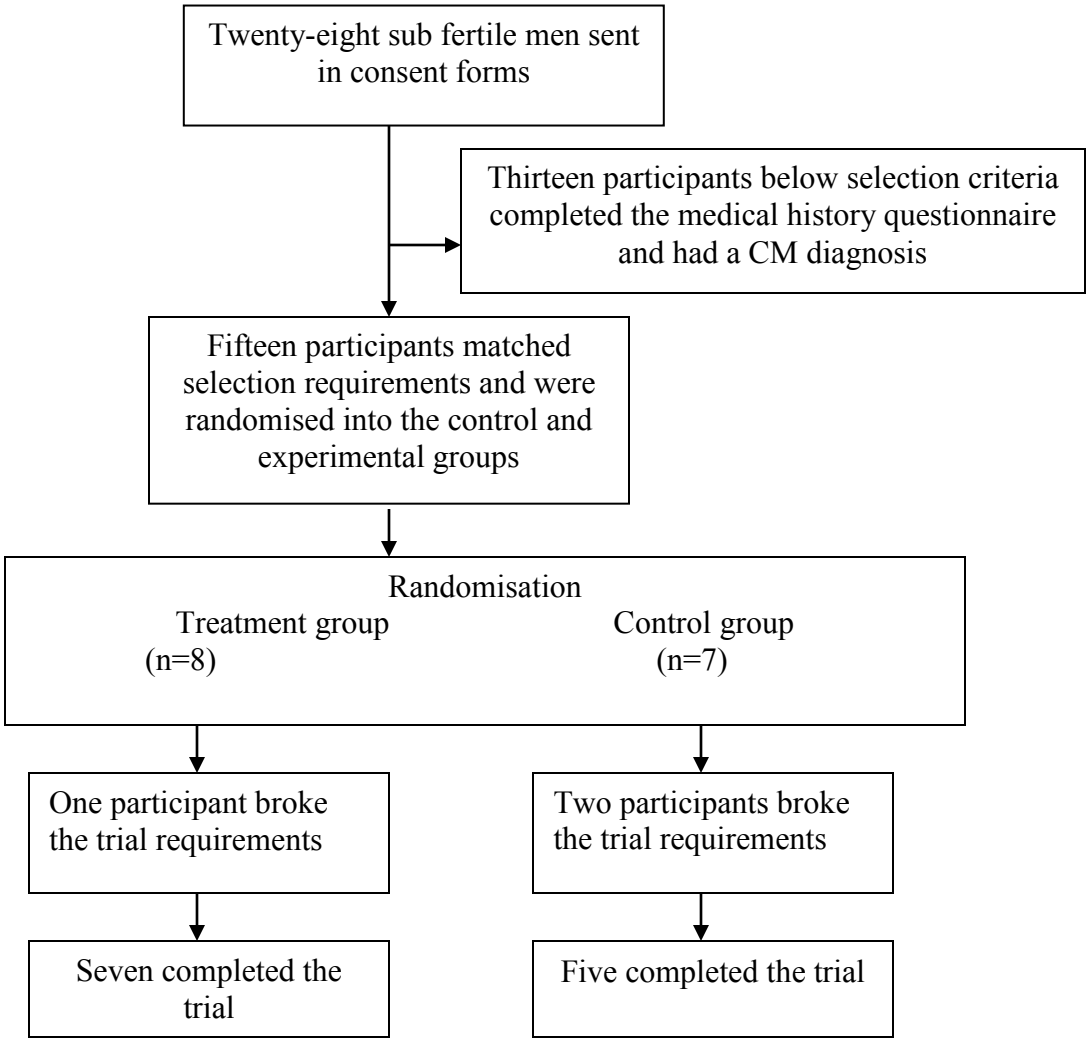

Figure 4.1 Flow chart for participant allocation.

4.1.1 Participant age

Participants randomised into the treatment and control groups were similar in age. The mean age of the treatment group was 42.3 years while the control group was 46.0 years.

	Age in years (SD)
Treatment n= 8	42.3 (7.6)
Control n= 7	46.0 (7.1)

Table 4.1 Mean age of the randomised group

4.2 Ethics application

The Human Research Ethics Committee at the University of Technology, Sydney (UTS) gave ethics approval prior to commencing the study. All documents related to the study, including all advertising, information to potential participants, handouts, consent forms and questionnaires were given ethics approval.

4.3 Recruitment

4.3.1 The recruitment method

UTS, has a media department that supports liaison with media groups such as local and interstate newspapers, radio stations and television channels. A media release concerning the study (appendix 3) was sent to all relevant media outlets. As a result, a total of four radio and seven newspaper interviews were conducted. Several major newspapers such as the Sydney Morning Herald and the Daily Telegraph wrote articles on the study based on the media release alone.

An internet web site was developed to support recruitment and a page titled 'Clinical trial' (appendix 4) was designed to provide information to interested men. Eligible men were offered the opportunity to discuss the study with the researcher via email and download helpful information such as further reading on the background of the CM treatment of male sub fertility, the consent form (appendix 5) and participant information

form (appendix 6). A telephone contact number was also provided in case the participant preferred contact through this channel. Communication through email allowed the researcher daily contact with interested parties while still providing a level of confidentiality to the enquiries. This was considered important in view of the sensitive nature of the research subject.

Internet links were made with related fertility support groups and professional organisations, such as ACCESS, the Australian national, non profit support network for infertile and sub fertile couples, as well as several other fertility support groups and on-line newsletters. An announcement about the study and a recruitment brochure was placed on these sites (appendix 7) and a paper on the subject of male infertility and Chinese medicine was also published on an online fertility support journal.

Letters introducing the study were sent to all Urologists based in Sydney (appendix 8) requesting support for the trial by allowing the researcher to place brochures in the waiting room of the Urologist. A request was also made for the Urologist to personally recommend eligible men to join the study.

Information about the study was sent to all known holistic medical practitioners or medical practitioners who had previously referred patients to the researcher. Telephone contact requesting support for the study was made to all Chinese medicine practitioners in the Sydney Yellow pages.

Ethics agreement from the Family Planning association of NSW was gained so that brochures about the study could be placed in three Family Planning centres around Sydney. Brochures were also left in the waiting room of the post-graduate student researcher.

4.3.2 Selection of participants to be randomised

Selection was based on sub fertile semen parameters recommended by the World Health Organisation (WHO) (Braude and Rowell, 2003b; World Health Organisation, 1992;

World Health Organisation, 1999) and research conducted by the teams lead by Coetzee and Kruger (Coetzee et al., 1998; Kruger and Franken, 2004) as discussed in Chapter two. Selection for the randomised clinical trial excluded men:

- Whose semen morphology was less than four percent normal;
- Who had sperm antibodies;
- Who had varicocele;
- Who lived more than sixty minutes away from the lab analysing semen samples. (This was a quality control measure to ensure sperm tested were alive and viable at the time of testing as the semen samples needed to be collected at the participant's home and delivered to the laboratory within the hour);
- Who had a semen analysis report more than 12 months old (All participants were given the opportunity to have another analysis undertaken to ensure their eligibility);
- Whose semen analysis results were not conducted by a pathology laboratory that specialised in male sub fertility;
- Whose morphology was greater than 15 percent normal forms;
- Who were younger than 18 and older than 60 years of age.

4.4 Randomisation

4.4.1 Identification numbers and randomisation

Once consent forms and semen analysis reports were received and participants' eligibility was confirmed, an Identification number (ID) was given to the participant. This ID number was placed on the consent form, the medical history questionnaire and semen analysis reports, collected during the study period, in order to preserve confidentiality and 'blinding' of the lab scientist measuring outcomes. Only the researcher, (Jann Mehmet) was aware of the name of the participants, their allocation into the control or treatment group and their ID numbers. A list of coded ID numbers matching participants' names was kept in a locked filing cabinet assessable only to the researcher.

Once ID's were allocated, the eligible participants were then matched into pairs and randomised into either the control or treatment groups through computer generated randomisation of their ID numbers. The participants were then notified by the researcher of their selection through either email or telephone and offered a start date option. The participants selected to join the randomised clinical trial needed to be available to join the study between June 26th 2004 and October 2nd 2004.

4.4.2 Randomisation into the control group

The participants allocated into the control group were required to provide two semen samples. The importance of the control group was to analyse their semen samples before the clinical trial started and eight weeks later to compare changes against the treatment group. When selected into the control group, participants provided a semen sample on a Saturday between June 26th and October 2nd 2004 and a Saturday eight weeks later. Once the participants in the control group provided their second semen sample at the end of the eight week control period they were offered free acupuncture and Chinese medicine treatments for eight weeks. This was to thank them for participating in the study and was also an ethical requirement.

4.4.3 Randomisation into the treatment group

The treatment group received both acupuncture and Chinese herbal medicine during the study. The effectiveness of the acupuncture and Chinese herbal medicine treatment was measured by comparing two semen analysis results. The first semen sample was taken just before the treatment began and the second eight weeks later, at the end of the treatment period. If selected into the treatment group, participants needed to be able to attend eight consecutive treatments of acupuncture. Participants were also required to provide a semen sample before the treatment commenced and eight weeks later. (Start time between June 26th 2004 and October 2nd 2004) The first consultation and treatment started on the day the first semen sample was collected or the following day which was a Sunday at the Rozelle Acupuncture and Chinese Medicine Centre. The following seven treatments were offered weekly.

4.5 The pragmatic treatment intervention

A pragmatic treatment approach was chosen rather than an explanatory randomised controlled trial. Pragmatic trials incorporate some key features of an explanatory trial; the patients are randomly allocated to groups and comparisons are made between the groups in terms of the measured response to treatment. In pragmatic trials however treatment conditions are kept as close as possible to how they would be delivered in day to day practice.

The pragmatic treatment, tailored to each individual in the treatment group was based on the collection of specific data. The data documented included:

- A medical history questionnaire (appendix 9);
- All eight treatments, including changes in symptoms;
- All herbal formulas prescribed;
- Batch numbers of single herbs used;
- A five gram sample labeled with the date and ID of the participant for each new formula prescribed.

4.5.1 The acupuncture intervention

The researcher used single-use, copper handled, (Viva) pre-packed sterile stainless steel needles without guide tubes. Sizes used were 13mm x 0.22 mm, 25 mm x 0.22 mm and 50 mm x 0.25 mm.

Insertion was approximately 5 mm for limb and abdominal areas, approximately 10 mm and horizontally inserted for upper back and perpendicular insertion for lower lumbar locations and approximately 40 mm in depth for gluteal insertions. The needles were inserted and left in place for a period of 25 minutes. The pulse was monitored to note changes and acupuncture points were selected based on achieving the required change in the pulse, signaling a positive benefit to the participant.

The specific acupuncture points chosen for each individual were documented during every treatment session. Acupuncture points chosen on the day, depended on the participant's initial diagnosis and the presentation of tongue, pulse, body palpation and presenting symptoms on the day of treatment. Acupuncture points along the bladder meridian were only used if muscular skeletal pain was identified in the lower back or other areas of the back including neck, thoracic or gluteal. Alternatively the researcher preferred using abdominal and limb acupuncture points if no muscular skeletal conditions existed.

General acupuncture points used to support fertility were CV4 and CV6 and KI16. These acupuncture points were always used as a 'branch' treatment to address the sub fertility in conjunction with other acupuncture points specific to the health of the participant or the 'root' of the complaint.

Acupuncture points to treat liver qi stagnation with digestive problems were: LR3; LI4; and ST25. These acupuncture points were frequently removed after 20 minutes and then a new selection of needles were inserted if there were chronic infections. For example, LU7, LU5, ST36, ST40, CV17 and KI27 for chest infection; LU7, LI 1, ST9, SI17, ST36, TE5 and KI4 for throat infection and acute viral infection with lowered immunity; LI4, LU7, BL2 and LI 20 for hay fever and sinusitis; LU7, LI11, ST36, ST37 and ST25 for loose stools; PC6, LR2, LR3, SP6, ST36, CV17, ST21 and ST25 for reflux.

4.5.2 Chinese medicine herbal intervention

The herbal formulas prescribed for each participant were selected based on the diagnosis of nine major Chinese medicine categories. These categories were:

Liver Qi Stagnation
Damp (Heat and cold)
Kidney Yin Vacuity
Kidney Yang Vacuity
Qi Vacuity

Blood Vacuity
Blood Stasis
Parasites
Liver Toxicity

Predicting the exact health profile of all the participants before the trial commenced was not possible. It was also anticipated that there would be changes in the signs and symptoms of the participants over the eight week treatment period, which would require modifying herbal prescriptions. Therefore, a selection of single CM herbs was collected based on the expected range of syndrome categories. A clinical trial dispensary was designed and established at the Rozelle Acupuncture and Chinese Medicine Centre. Single herbs were donated by a supplier listed with the Therapeutics Goods Administration (TGA). A patent formula specifically designed to improve sperm count was also donated to the trial and used when relevant.

4.5.3 Chinese medicine herbal formulas

1. Liver Qi Stagnation

Da Chai Hu Tang (Major Bupleurum Decoction)
Xiao Chai Hu Tang (Minor Bupleurum Decoction)
Jia Wei Xiao Yao San (Augmented Rambling Powder)
Xiao Yao San (Rambling Powder)
Si Ni San (Frigid Extremities Powder)

Variations included:
Qi Gi San (Open the Diaphragm Powder)
Bao He Wan (Preserve Harmony Pill)
Hua Gan Jian (Transform the Liver Modified)

2. Damp

Long Dan Xie Gan Tang (Gentiana Long Dan Cao Decoction to Drain the Liver)
Ba Zheng San (Eight-Herb Powder for Rectification)

San Huang Xie Xin Tang (Coptis and Rhubarb modified)

Xie Xin Tang (Drain the Epigastrium Decoction)

Ban Xia Xie Xin Tang (Pinellia Decoction to Drain the Epigastrium)

Ban Xia Hou Po Tang (Pinellia and Magnolia Bark decoction)

Liu Jun Zi Tang (Six Gentlemen Decoction)

3. Kidney Yin Vacuity

Zuo Gui Wan (Restore the Left Pill)

Zi Yin Zhong Zi Wan (Nourish Yin to Enhance Fertility Pill)

Variations included:

Yi Guan Jian Tang (Linking Decoction)

Mai Men Dong Tang (Ophiopogon Decoction).

Zhi Bai Di Huang Wan (Annenmarrhena Phellodendron and Rehmannia pill)

4. Kidney Yang Vacuity

Jing Si Yu Tang (Boost the Essence Birthing Decoction)

5. Qi Vacuity

Wu Zi Yan Zong Wan (Men's Treasure Pills)

Ba Zhen Tang (Eight Treasure Decoction plus Additions)

Variations included:

Xiang Sha Liu Jun Zi Tang (Six Gentlemen Decoction with Aucklandia & Amomum)

6. Blood Vacuity

Si Wu Tang (Four Substance Decoction)

Variations included:

Lui Wei Di Huang Wan (Six Ingredient Pill with Rehmannia)

7. Blood Stasis

Tong Jing Jian Tang (Free Jing Decoction)

Xu Fu Xue Yu Tang (Drive out Stasis in the Mansion of Blood Decoction)

8. Parasites

Zhui Chong Wan (Pursue Parasites Pill)

9. Liver Toxicity as a result of environmental factors

The last category was newly developed during the present study and further clarification of the signs and symptoms related to 'Liver toxicity' is provided in Chapter 6 (Discussion). As previously stated, CM has limited discussion or experience in the area of environmental contaminants. Therefore, all single CM herbs listed are recommended conservatively, as a need for more specific research is required. The single herbs requested included:

Pu Gong Ying, Herba Taraxaci Mongolici cum Radice (Dandelion)
Tu Fu Ling, Rhizome Smilacis Glabrae (Smilax)
E Zhu, Rhizoma Curcumae E zhu (Zedoria)
Yu Jin, Tuber Curcumae (Curcuma)
Hei Zhi Ma, Semen Sesami Indici (Black Sesame Seeds)
Bai Hua She She Cao, Herba Hedyotidis Diffusae (Olendandia)
Ban Zhi Lian, Herba Scutellariae Barbatae (Herba Scutellariae Barbatae)
Guo Luo, Fructus Trichosanthis (Trichosantes Fruit)
Chai Hu, Radix Bupleuri (Bupleurum)
Bai Shao, Radix Paeoniae Lactiflorae (White Peony)
Huang Qin, Radix Scutellariae Baicalensis (Scute)

4.6 Measuring outcomes

Changes in semen count, motility and morphology were analysed by semen analysis before the treatment and control period and eight weeks later at the end of the treatment

and control period. The scientist (James Leong) analysing the semen samples remained 'blind' to participant status.

4.6.1 Analysing semen samples

Semen samples were obtained and stored in sterile plastic containers that were delivered to the laboratory within one and a half hours of ejaculation. The sample was then centrifuged at 600g for 15 minutes. Ten µl of semen was then placed on a sterile slide. A phase-contrast microscope with 400 × magnification was used to scan the slide and estimate count and motility. Then 5-10µl of the solution was placed on a sterile glass slide and stained with Papanicolaou stain which allows for the examination of morphology (the acrosomal and post-acrosomal regions of the head, the cytoplasmic droplet, the mid-piece and the tail). Classification was based on strict criteria and then frequencies were transcribed onto the semen analysis report.

4.6.2 Statistical analysis

The data were entered into Minitab, Windows version 14. An analysis of variance (using the general linear model) was used with the response equal to the post measurements, the model equal to the group (treatment or control) and the pre measurements entered as a covariate. The residuals were checked for normal errors using the Anderson-Darling test, and for equal variances using Bartlett's test.

Chapter V: Results

5.1 Data analysis of the effect of Chinese Medicine (CM) on semen morphology, motility and count.

The following analysis was performed using only those participants in the treatment group (n= 8) and the control group (n =7).

The two groups were compared to determine any significant differences in the baseline measurements of morphology, motility and count. Table 5.1 shows that the mean for the treatment group is lower for each of the three sperm parameters, but none of these differences were statistically significant. Subjects in both groups had a similar age, with the mean age of the treatment group 42.3 years and the mean age of the control group 46.0 years.

Variable	**Treatment mean (SD)** n=8	**Control mean (SD)** n=7
Morphology (% normal forms)	12.6 (7.1)	9.4 (4.0)
Motility (%)	150.3 (66.3)	135.6 (59.4)
Count (million)	180.2 (109.1)	129.5 (95.6)
Age (years)	42.3 (7.6)	46.0 (7.1)

Table 5.1 Baseline mean of morphology, motility, count and age of the two groups.

5.1.1 Morphology

Prior to analysis, an Anderson-Darling test for normality of residuals and the Bartlett's test for homogeneity of variance were undertaken to check for normal distribution of data.

The data were found to be normally distributed (p values of 0.5 and 0.2 respectively). Therefore the assumptions for ANOVA were satisfied.

From Table 5.2 and Figure 5.1 we can see that on average participants in the control group experienced a decrease in the mean percentage of normally formed sperm (9.4 to 8.4 %), while the treatment group had an observable increase in normal forms (12.6 to 20.3 %).

Prior to treatment, the mean percentage normal forms were 12.6 (range 2 % to 25 %). Following treatment there was a statistical increase to 20.3 (range 11 % to 36 %). No statistical change in mean percentage normal forms was observed for the control group with a decrease from a mean 9.4 (range 3 % to 16 %) to 8.4 (range 2 % to 15 %). A statistical difference was seen between the two groups ($p<0.04$) in terms of morphology.

Group	**Pre mean (SD) (% Normal forms)**	**Post mean (SD) (% Normal forms)**
Treatment n= 7	12.6 (7.1)	20.3 (9.3)
Control n=5	9.4 (4.0)	8.4 (4.7)

Table 5.2 Mean morphology scores before and after the eight week treatment and control period.

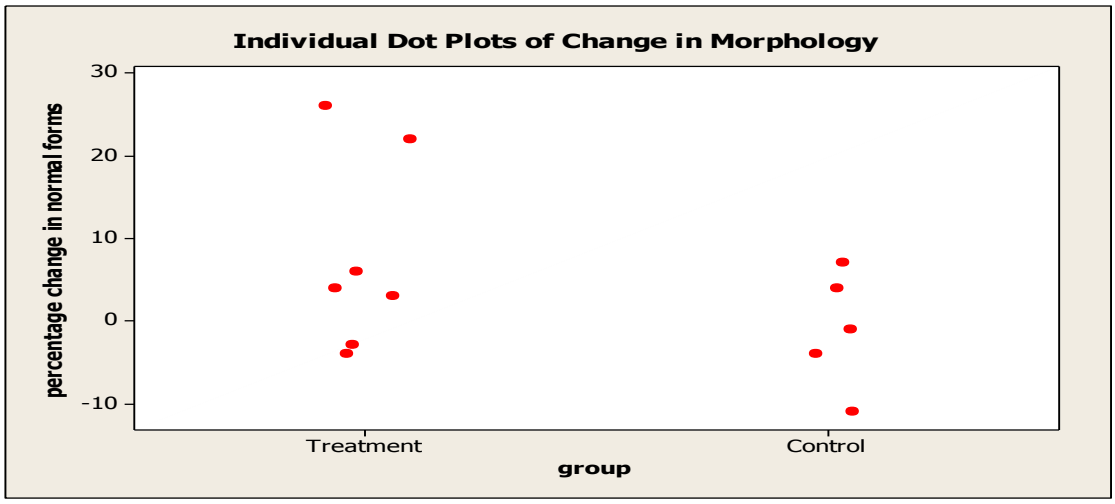

Figure 5.1 Dot plots of change in morphology (morphology post minus morphology pre).

5.1.2 Motility

Prior to analysis an Anderson-Darling test for normality of residuals and the Bartlett's test for homogeneity of variance were undertaken to check for normal distribution of data. The data were found to be normally distributed (p values of 0.6 and 0.6 respectively). Therefore the assumptions for ANOVA were satisfied.

Prior to treatment, the mean percentage motility score was 150.3 (range 40 % to 217 %). Following treatment there was a statistical increase to 169.3 (range 45 % to 250 %). No statistical change in mean percentage motility was observed for the control group with a mean decrease of 135.6 (range 50 % to 210 %) to 84.0 (range 20 % to 130 %).

From Table 5.3 it was observed that on average participants in the control group experienced a drop in motility (135.6 % to 84.0 %), while the participants in the treatment group experienced a modest increase in motility (150.5 % to 169.3).

A statistical difference was seen between the two groups ($p<0.008$) in terms of motility.

Group	Pre mean (SD) (% Motility)	Post mean (SD) (%Motility)
Treatment n=7	150.3 (66.3)	169.3 (71.2)
Control n= 5	135.6 (59.4)	84.0 (43.9)

Table 5.3 Mean motility scores before and after the eight week treatment and non treatment period.

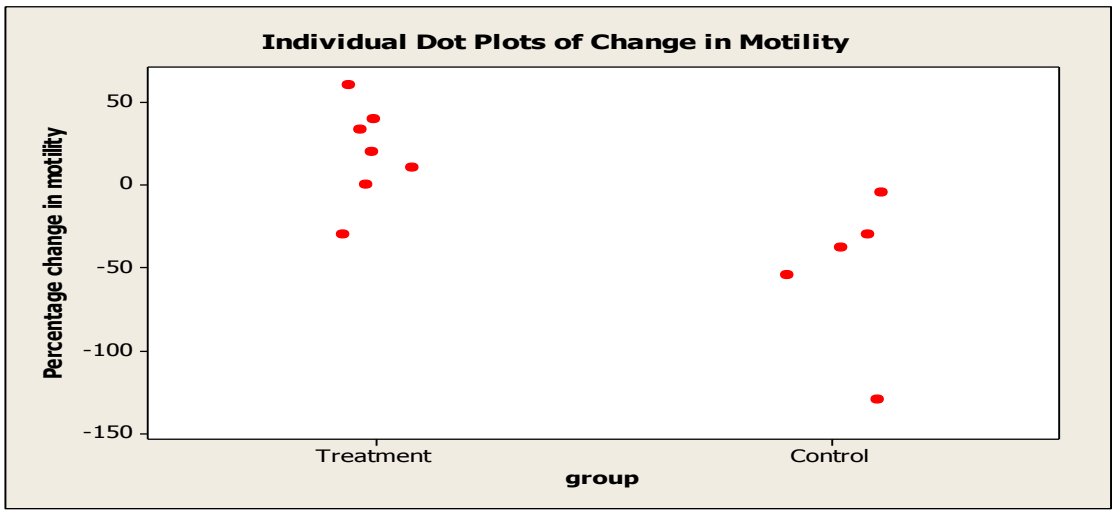

Figure 5.2 Dot plots of change in motility (motility post minus motility pre).

5.1.3 Count

Prior to analysis an Anderson-Darling test for normality of residuals and the Bartlett's test for homogeneity of variance were undertaken to check for normal distribution of data. The data were found to be normally distributed (p values of 1.0 and 0.7 respectively). Therefore the assumptions for ANOVA were satisfied.

Prior to treatment, the mean count was 180.2 million (range 68.4 million to 373 million). Following treatment there was a statistical increase to 323.9 million (range 81 million to 826 million). No statistical change in mean count was observed for the control group with

a decrease from a mean of 129.5 million (range 38.1 million to 235.2 million) to 115.1 (range 7 million to 294 million).

From Figure 5.3 we can see that only two participants in the control group experienced an observable increase in sperm count, but in the treatment group five of the seven participants experienced an increase.

No statistical difference was seen between the two groups ($p<0.1$) in terms of count.

Group	**Pre mean (SD)** **(Total count in millions)**	**Post mean (SD)** **(Total count in millions)**
Treatment n=7	180.2 (109.1)	323.9 (250.3)
Control n=5	129.5 (95.6)	115.1 (112.2)

Table 5.4 Mean sperm count in the treatment and control groups before and after the eight week treatment and control period.

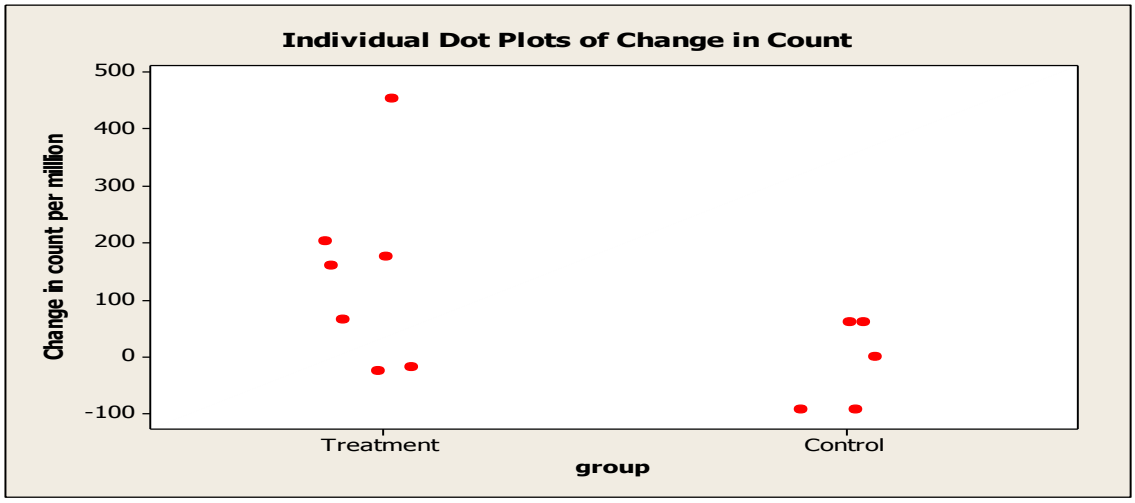

Figure 5.3 Dot plots of change in count (count post minus count pre).

Post hoc analysis was conducted on the changes in sperm count within the treatment group. There was an observed increase in sperm count for five participants in the

treatment group who received supplementation with a Chinese herbal formula designed to increase count, with a mean increase in sperm count of 220 million sperm. The mean increase (220 million sperm) was clinically significant.

Notably, the two participants in the treatment group who did not receive Chinese herbal supplement to increase sperm count, experienced a mean decrease in count of 26 million sperm in their semen samples after the treatment period. Both participants presented with chronic infections and these infections were treated with Chinese herbal formulas which contain herbs which have an antibacterial affect. Chinese medicine protocol dictates that all infections must be treated and resolved before any supplementing formulas can be prescribed.

	Given herbal tonics	**Not given herbal tonics**
Treatment Group (n=7)	5 (71.4%)	2 (28.6 %)
Mean change in count	+ 220 million	- 26 million
Range	63.6 to 826 million	-19 to -34 million

Table 5.5 The distribution of subjects who received tonics.

Five participants in the treatment group were given Chinese herbal formula and acupuncture treatment for a chronic infection during the trial period. Two participants in the treatment group given Chinese herbal formula for chronic infection were also given Chinese herbal supplements to increase sperm count once their infections were resolved. These two participants in the treatment group had clinically significant increases in their semen count (mean average increase of 313.5 million) compared to the two participants in the treatment group that received no Chinese medicine herbal tonics to improve semen count (mean decrease of minus 26 million).

Group	Herbal treatment for infection
Treatment (n=7)	5(71.4%)
Control (n= 5)	4 (80.0%)

Table 5.6 Participants who received herbal remedies for chronic infection during the study.

5.2 Case history results

Twenty eight sub fertile men sent in consent forms. While only 15 of this sub fertile group matched the selection criteria required to participate in the randomised clinical trial, all 28 sub fertile participants completed the medical history questionnaire. The medical history questionnaire profiled significant common characteristics of the sub fertile group, based on CM categories and specific medical diagnosis.

5.2.1 Medical history

The mean time for attempting conception was 4.3 years as shown in Table 5.7. Twenty-one (75%) participants responded to this question, while seven (25%) participants did not.

Despite this relatively long time span for attempting conception, of the 28 participants, only two (7.1%) men had been referred to an urologist for further testing and investigation before the current study. Both men were diagnosed with varicocele.

During the duration of this study, 13 (46.4%) of the 28 participants were referred to an urologist that specialised in male fertility for further investigation because of their high risk for varicocele and testicular cancer. The participants at risk had normal forms below five percent and were of the group excluded from the randomised clinical trial. The other 15 (53.6%) participants, who met the selection criteria for the randomised clinical trial, while sub fertile, were not considered at risk of varicocele or testicular cancer because

their normal forms were above five percent. Of the 13 men at risk, 12 (92.3%) men acted upon the advice given by the researcher and visited an urologist and seven (58%) were found to have a varicocele, while no men were diagnosed with testicular cancer.

Time attempting conception n= 21	**Referred to a Urologist before the trial** n= 28	**Varicocele diagnosed before the trial** n= 28	**Investigation for varicocele recommended** n= 28	**Varicocele diagnosed during trial** N= 12
4.3 years Range (2-8)	2 (7.1%)	2 (7.1%)	13 (46.4%)	7 (58%)

Table 5.7 The medical history of participants in the study.

5.2.2 History of chronic infection and tiredness

An infection was considered chronic if it had been ongoing and continuous for over three months. Of the 28 participants, 23 (82.1%) responded to the question regarding chronic infection while five (17.8%) participants did not. Of the 23 participants, 12 (52.1%) reported having a chronic infection. The most common infection reported was a chronic chest infection that also presented as shortness of breath, breathing difficulties, coughing, asthma and tiredness.

Hay fever and sinusitis infection were considered chronic if the incidence was over a three month period directly before the trial period. Of the 28 participants, 23 (82.1%) responded to this question and five (17.8%) did not. Of the 23 participants, 16 (69%) were found to have chronic hay fever and sinusitis.

A participant was considered chronically tired if he had been experiencing this condition for more than three months leading up to the current study. Of the 28 participants, 23 (82.1 %) responded to this question while five (17.8%) did not respond. Nineteen (82.6%) men complained of chronic tiredness and this group was more likely to experience colds

and flu at least once a month, sleeplessness, anxiety and over work (working more than 12 hours a day, six days a week).

General infection n= 23	Hay fever and sinus infection n= 23	Tiredness n=23
12 (52.1%)	16 (69.5%)	19 (82.6%)

Table 5.8 The incidence of general chronic infection; chronic hay fever and sinusitis; and chronic tiredness.

5.2.3 History of significant exposure to environmental or work place contaminants

Twenty-eight participants were asked about their exposure to environmental contaminants. Five (17.8%) participants did not respond. Of the 23 (82.1%) participant responses, nine (39.1%) experienced significant exposure to environmental contaminants while seven (30.4%) also complained of tenderness around the liver when palpated during diagnosis. It was observed that the participants most likely to have a past history of environmental contaminant exposure were the sub fertile men with normal forms below five percent.

Exposure to environmental contaminants n= 23	Liver tenderness n= 23
9 (39.1%)	7 (30.4%)

Table 5.9 The incidence of significant and potentially detrimental exposure to environmental and work place contaminants and liver tenderness on palpation during CM diagnosis.

5.2.4 History of testicular symptoms

Twenty-eight participants were asked about testicular pain, history of undescended testes and history of a significant injury to the testes. Five (17.8%) participants did not respond.

Of the 23 (82.1%) participant responses, six (26 %) complained of intermittent testicular pain and of this group four (66%) had normal forms below five percent and therefore below selection criteria to participate in the randomised trial.

Only one (4.3%) participant had a history of undescended testes and this participant had normal forms below five percent and was not eligible to participate in the randomised trial.

Four (17.3%) participants had experienced a significant impact to the testes. All of these participants had normal forms below five percent and therefore were not eligible for the randomised clinical trial.

Pain in the testes	**Pain in men below selection criteria**	**% of men with pain in testes below selection**	**History of undescended testes**	**Significant injury to the testes**
n= 23	n=10	n=6	n=23	n=23
6 (26%)	4 (40%)	4 (66%)	1 (4.3%)	4 (17.3%)

Table 5.10 Testicular medical history.

5.2.5 Chronic general symptoms important in CM diagnosis

Twenty-eight participants were asked questions for a range of general symptoms that are diagnostically significant for CM. Symptoms were considered chronic if they occurred for over a three month period leading up to the participation in the current study. Five (17.8%) participants did not respond. Of the 23 participant responses, eight (34.8%) experienced chronic night sweats; 14 (60.8%) experienced chronic irritability of varying degrees; 16 (69.5) experienced chronic anxiety; 14 (60.8%) experience regular loss of sleep: four (17.4%) were on medication for chronic conditions; eight (34.8%) had a history of psoriasis on various parts of the body with varying severity and eight (34.8%)

men also experienced regular bouts of fungal infection and itchiness and irritation around the groin and testes.

A high number of participants experienced digestive complaints, with 18 (78.2%) men experiencing chronic loose stools and 15 (65.2%) men experiencing chronic reflux. Eleven (47.8%) men experienced chronic lower back pain.

Night sweats n= 23	**Irritability** n= 23	**Chronic anxiety** n=23	**Chronic loss of sleep** n= 23	**Long term medicated** n=23
8 (34.8%)	14 (60.8%)	16 (69.5%)	14 (60.8%)	4 (17.4%)

Chronic loose stools n=23	**Chronic reflux** n= 23	**Chronic lower back pain** n=23	**Groin fungal infection** n=23	**History of psoriasis** n=23
18 (78.2%)	15 (65.2%)	11 (47.8%)	8 (34.8%)	8 (34.8%)

Table 5.11 The incidence of chronic general health complaints

5.4 Summary of responses from the feedback form (appendix 10)

5.4.1 Recruitment

All 15 participants randomised into the control and treatment groups were given a feedback form. Fourteen (93.3%) participants completed the form and the researcher was blind to the participant's identity.

5.4.2 Q 1a: Did the web site or initial communication and information via emails assist you in feeling more comfortable about making enquiries about the trial? Q1b:

Was the information on the web site or communication via emails helpful in making you decide to join the trial?

Seven (50%) of the 14 participants did not use the internet. Those who did use the internet found the web site and email communication helpful or very helpful in making enquiries about the trial and helping them decide to join the trial.

Response	Web site and emails helpful re enquires? (n=14)		Web site and emails helpful re joining the trial? (n=14)	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Did not use the internet	7	(50.0)	7	(50.0)
Helpful	4	(28.0)	5	(35.7)
Very helpful	3	(21.0)	2	(14.3)

Table 5.12 Participants' response concerning the helpfulness of email and web site communication in supporting their decision to join the study.

5.4.3 Q 2: Did you find the trial well organised?

All participants rated the organisation of the trial to be good or very good.

Response	Rate the organisation of the trial? (n=14)	
	Frequency	**Percentage**
Good	4	(28.6)
Very good	10	(71.4)

Table 5.13 Participants' response concerning the organisation of the trial.

5.4.4 Q 3: Did you have any apprehensions about working with a female practitioner? Q 4: Do you think a male practitioner would be more suitable?

None of the participants had any apprehensions about working with a female practitioner. None of the participants responded 'yes' to "Do you think a male practitioner would be more suitable?" with ten (71%) participants answering 'no' and four (28.6%) 'not sure'.

Response	Suitability of a female practitioner? (n=14)	
	Frequency	**Percentage**
Not a problem	10	(71.4)
Not sure	4	(28.6)

Table 5.14 Participants' response concerning the suitability of a female practitioner in treating sub fertile men compared to a male practitioner.

5.4.5 Q 5: Have you received acupuncture before the trial? Q 6: Have you received Chinese herbal medicines before the trial?

Eight (57.1%) of the participants had received acupuncture before the trial and seven (50%) had taken Chinese herbal medicines before the trial. All 14 participants would consider having acupuncture or Chinese herbal medicine treatments in the future.

Response	Received acupuncture before the trial (n=14)		Received Chinese herbs before the trial (n=14)	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Yes	8	(57.1)	7	(50.0)
No	6	(42.9	7	(50.0)

Table 5.15 Participants who received acupuncture and Chinese herbal medicine before the trial.

5.4.6 Q 7: Would you consider having acupuncture or Chinese herbal medicine treatments in the future?

All 14 participants said 'yes' to considering having Chinese medicine treatment in the future.

Response	Consider acupuncture and Chinese herbal medicine in the future? (n=14)	
	Frequency	**Percentage**
Yes	14	(100)
Not sure	0	(0.0)
No	0	(0.0)

Table 5.16 Participants' response considering acupuncture and Chinese herbal medicine treatment in the future.

5.4.7 Q 8: Rate the benefits to your health as a result of your participation in the trial.

Thirteen (92.8 %) of the 14 participants rated the benefits to their health as either 'good' or 'better' as a result of their participation in the trial.

Response	Benefits of participating in the trial? (n=14)	
	Frequency	**Percentage**
None	1	(7.1)
Good	6	(42.9)
Very good	5	(35.7)
Excellent	2	(14.3)

Table 5.17 Participants' response considering the benefits of participating in the trial.

5.4.8 Q 9: Are you glad that you participated in the trial?

All participants were pleased or very pleased that they participated in the trial.

Response	Glad you participated in the trial? (n=14)	
	Frequency	**Percentage**
Pleased	4	(28.6)
Very pleased	10	(71.4)

Table 5.18 Participants appraising their involvement in the trial by answering the question, are you glad you participated in the trial?

Chapter VI: Discussion

6.1 Results of the randomised clinical trial

Despite the small participant numbers, this pilot study has demonstrated that acupuncture and Chinese herbal medicine can statistically increase both sperm morphology and motility. This increase was statistically and clinically significant.

Morphology

For morphology, the treatment group experienced a statistically better result than the control group with the longitudinal analysis of the primary outcome showing a significant difference between the treatment and control group ($p<0.04$) eight weeks after randomisation. Changes were also consistent with a clinically significant improvement in morphology.

Each sub fertile participant in the treatment group was provided with eight pragmatic Chinese herbal medicine and acupuncture treatments. Even so, clear patterns emerged amongst the randomised group. Of particular interest was the high incidence (71.4%) of participants in the treatment group who received CM for a chronic infection. Therefore, chronic infection may influence morphology in a high percentage of sub fertile men and CM treatment may lead to significant improvement in both morphology and chronic infection. Whether or not certain acupuncture points or a combination of points or Chinese herbal formulas specifically influenced these factors, was not examined, given the pragmatic design.

Motility

Compared with the control group, acupuncture and Chinese herbal medicine improved semen motility, with the primary outcome measuring a significant difference between the treatment and the control group ($p<0.008$) eight weeks after randomisation.

Count

The differences for count were not statistically significant, but the trend for the two groups was in the expected direction, with the treatment group achieving a increase in count better than the control group. For example, Figure 5.3 shows that only two participants in the control group experienced a small increase in count, but in the treatment group the results were varied, with five out of the seven participants experiencing an increase in count that was clinically significant. Two men in the treatment group did not improve.

A possible cause for the variation in count may be related to the number of participants in the treatment group who actually received CM herbal supplements to improve count. For example, from Table 5.5 we can see that only five of the seven participants in the treatment group received Chinese herbal supplements designed to improve sperm count. Of interest, only three of these men received these particular supplements for the whole eight weeks, because the other two participants who received supplements to improve count also required Chinese herbal supplements for a chronic infection throughout the treatment period. Despite this, all five participants in the treatment group receiving Chinese herbal supplements to increase sperm count had a clinically significant increase in sperm count (mean 220 million sperm). The three participants in the treatment group who received the supplement for the whole eight weeks, experienced the greatest increase in total sperm count (a post treatment sperm count of 826, 427.5 and 516 million). Therefore in all cases when Chinese herbal formulas were used to improve count, count increased to a clinically significant degree. This was particularly obvious when Chinese herbal formulas to supplement count were used for the whole eight week period of the study.

On the other hand, the two participants in the treatment group who did not receive supplements to improve sperm count at all during the eight week treatment period, (because they were given Chinese medicine supplements to treat a chronic infection), did not see improvements in their count. Clearing infection before providing supplementation

is an important treatment protocol in Chinese medicine. Importantly, improvements in morphology were observed as a result of this protocol.

Control group count

One of the participants in the control group broke the trial requirements during the control period by self prescribing Chinese medicine herbs that supplement semen count. This was achieved by visiting a Chinese medicine grocery store in Sydney's Chinatown and requesting a patent herbal supplement to support male fertility. Participation in the study had motivated the man to actively try and help his condition and consequently his sperm count increased from 37 million to 100 million during the eight week control period. This improvement in count was clinically significant and may also suggest that Chinese herbal supplement for increasing sperm count is effective. Of interest, while achieving improvement in count, this participant did not see improvement in morphology.

Several of the men in the control group reported that they started to exercise regularly and limit their consumption of alcohol and junk food during the control period. This behavior was contrary to agreed guidelines as outlined in the consent form, where changes to diet and lifestyle were discouraged in order to control extraneous variables. Despite the changes in exercise and diet, no participant achieved significant improvement in their semen parameters.

The control groups' behavior suggests that keeping participants 'blind' to their selection is preferable to placing them on a wait list. As a pilot study in a new field of research, it was considered important to offer all participants an incentive to join the trial. It was perceived that if the participants were offered a potential placebo, they might not join the study in the first place.

Another possible criticism of the design concerns the 'wait list' control. Whilst a wait list provides a control for time tied factors such as 'regression to the mean' and 'spontaneous fluctuation', it does not control for placebo effects due to subject attention, expectation and beliefs. While the impact of these effects on the participants in the present study was

possible, the use of objective outcome measures such as sperm morphology, count and motility limit their effect. Indeed, placebo effects are more likely to occur when subjective measures are used, such as levels of pain and quality of life measurement. Due to the encouraging results of this study, it is recommended that in future, all studies on male sub fertility and CM if possible be placebo-controlled and participant-blinded.

6.2 Discussion on the important findings observed during the study

Twenty-eight sub fertile men completed a medical history questionnaire. Some interesting medical history patterns emerged and these findings are discussed below.

6.2.1 Varicocele

The results of the study suggest a much higher incidence of varicocele among the men at risk (58 %) than has been in the past suggested (> 37 %). Due to the high level of abnormal forms produced when there is a varicocele present, men are either directed towards varicocelectomy or IVF and ICSI. The success rates for varicocelectomy and IVF procedures are questionably low as indicated by the recent systematic review on varicocelectomy which suggests that there is no evidence that embolisation or ligation benefit male sub fertility (Evers and Collins, 2003) and the BEST report on IVF and ICSI cycles (which suggests a success rate of only 11.3%) (Min et al., 2004). Despite these results, both varicocelectomy and IVF continue in Australia. However, while previous research conducted on sub fertile men has measured the effect of acupuncture on improving ICSI fertilisation rates (Pei et al., 2005; Siterman et al., 1997; Siterman et al., 2000), there has been no research to show the effectiveness of acupuncture in supporting varicocelectomy.

Observing the effectiveness of CM treatment immediately after varicocelectomy was considered of interest, as there were a large number of men joining the study with undiagnosed varicocele. This was considered problematic because CM has no proven independent method to either diagnose or treat this condition. Consequently, an opportunity was seen to two match men with varicocele who then chose to undergo

corrective varicocelectomy and randomise them into the control and experimental groups. This is a good example of identifying a known variable and matching participants accordingly.

Two men were matched and randomised and the semen analysis of both participants was compared before surgery and after the treatment and control periods. Initial findings appear promising as the participant in the treatment group had a clinically significant improvement compared to the control participant (a pre trial morphology of 2 % normal forms and a post trial morphology of 28 % normal forms compared to a pre trial morphology of 2 % normal forms and a post trial morphology of 7 % normal forms for the control participant.) Due to the short duration of the study, it was not possible to match larger numbers of men undergoing varicocelectomy.

Based on these findings, it is considered that a large randomised double blind controlled study to measure the effectiveness of CM therapy in complementing varicocelectomy post operatively might be beneficial. Such a study might prove to be very important, as it appears that varicocele is a common problem and an effective treatment is not currently available. A large scale observational study also needs to be conducted to determine a more accurate estimate of the actual percentage of sub fertile men who have varicocele.

Chinese medicine discussion on varicocele

While Chinese medicine discussion on varicocele is limited, some authors suggest that a varicocele is mainly related to the CM pattern of blood stasis (Becker, 2000; Clavey, 2003; Ishikawa et al, 1996). In the present study, there appeared to be no evidence that blood stasis was the major cause of varicocele. Instead either the participant had a previous history of a serious impact to the testes that lead to local trauma (and this was indicated especially in men less than 35 years of age such as J109), or the participant exhibited a spleen not holding pattern with chronic loose stools and spleen qi vacuity. Not one participant diagnosed with varicocele complained of pain in the testes. On the other hand, when blood stasis is the cause of a condition, some occasional local pain, including sharp stabbing pain, should be expected. This was not reported by participants

diagnosed with varicocele. It is recommended that the CM protocols for diagnosing and treating varicocele be updated to incorporate protocols that are based on current evidence. Further research is suggested on the effectiveness of CM treatment on varicocele both independently and collaboratively with varicocelectomy.

6.2.2 Exposure to environmental contaminants

A percentage of participants (39.1%) had a previous history of significant exposure to known and dangerous environmental and work place poisons, such as agent-orange, lead and cleaning products (Table 5.9). Four of these men experienced normal forms below five percent.

Environmental factors are said to cause specific head defects associated with testicular stress acting on the sertoli cells during the later stage of spermatogenesis. In particular the tapered head abnormality specifically suggests environmental factors (Franken and Kruger, 2004a; Hofman and Haider, 1985; Menkveld et al., 1991). This particular head defect can be labeled mild, moderate or severe depending on the severity of the abnormality and the level of exposure. This is a very good example of the diagnostic usefulness of semen analysis. Mild, moderate or severe tapering may indicate mild to severe environmental damage, reflecting the treatment time necessary to improve semen parameters and general health. Severe tapering is thought to be irreversible (Hofman and Haider, 1985).

Coiled tails have also been associated with damage to sperm as a result of a man's exposure to organic solvents such as those found in glues, paints and cleaning fluids (Alexander, 1992; Franken and Kruger, 2004b). Coiled tails may also be caused by increases in scrotal temperature (Alexander, 1992).

Significantly the average ART laboratory analysing semen samples does not make clear and specific comments on the particular defects observed. This is quite unfortunate, as a more detailed report might enormously assist investigation and treatment. As a result this study suggests that an independent laboratory reporting more thoroughly on specific

semen defects is essential if a comprehensive understanding of factors contributing to male sub fertility is ever to be reached.

CM protocol on treating men with heavy metal contamination

As mentioned in chapter one, while environmental contaminants are acknowledged in CM journal articles as a cause of male sub fertility, no known method of diagnosis or treatment has been developed in CM. Therefore, the present study was interested in observing the participants with a history of environmental exposure and documenting specific common patterns. Once observed, this group was found to have three distinct CM signs. These signs included a specific tongue profile (very red around the tongue edges); pulse profile (a specific new pulse pattern, labeled 'toxic liver', related to significant chronic poisoning); and tenderness around the liver on palpation (and in more severe cases tenderness around the spleen).

To date, the main method used to identify risk of serious poisoning through heavy metal and chemical exposure is conducted by hair analysis. The forensic department at UTS has achieved some level of expertise in this area, using this technique to identify a slow poisoning over a long period of time. While the present study was not able to obtain a donation of laboratory hair analysis testing for participants at risk, those participants identified at risk volunteered to pay this fee to Interclinical Laboratories (the main laboratory analysing hair samples in Australia). Once analysed, hair samples provided some anecdotal evidence of the development of a CM pattern of heavy metal exposure because an association to pulse, tongue and palpation signs listed above were seen.

Of importance, during CM treatment in the study the overall general health of the participant with heavy metal contamination improved quite markedly, however the three specific signs observed (tongue, pulse and palpation) improved very slowly. This might be considered an indication of the chronic nature of the CM toxic liver pattern and the potential seriousness of the condition, indicating a more serious risk to the participants' long term health. Future collaborative work with a university forensic department might

prove valuable in identifying men at risk and developing an effective treatment to reduce that risk.

6.2.3 Low grade chronic infection and reduced immunity

Poor immune response and chronic infection were often present in the profile of the participants in the study. For example, Table 5.8 shows that a high percentage of the total number of participants experienced chronic sinusitis (69%). In the treatment group 71.4% of participants were treated for some form of chronic infection, while 80% of the control group experienced some form of chronic infection (Table 5.6). The other main patterns included 82.6 % of the total number of participants' who experienced reduced general immunity manifesting as chronic tiredness and continuous cold and flu episodes throughout the year (Table 5.8). The participants who experienced chronic tiredness were also more likely to suffer from chronic infections. In addition, 78.2% of participants experienced some form of digestive complaint such as loose stools and this might account for the reduced level of general immunity and increased incidence of tiredness and chronic infection. Furthermore, irritability was experienced by 60.8% of participants; and anxiety in 69.5 % of participants; night sweats in 34 % of participants; and chronic lower back ache in 47.8% of participants (Table 5.11).

A low grade testicular infection was diagnosed (as indicated by testicular pain) among participants with normal forms below five percent. Prior to the study, this condition remained undiagnosed and untreated within this group. This is important in light of earlier papers published more than 20 years ago that reported that several ART laboratories observed a high incidence of undiagnosed low grade testicular infection, believed to be an underestimated cause of male sub fertility (Hargreaves, 1988a). In clinical practice the bacteria related to chronic testicular infection are often referred to as 'super bugs' (Wong et al., 2000) and medication similarly prescribed to treat anthrax is given. Liver damage and hip fractures as a result of bone density loss are some of the serious side-effects of this medication. These medications have been prescribed because low grade testicular infection remains antibiotic resistant and treatment to date is unsuccessful (Aziz et al., 2004). As a result in the last 20 years this problem has generally

been overlooked and as a consequence these men go untreated and directed instead to IVF centres.

The treatment of a testicular 'super bug' remains both of interest and a challenge to CM protocol, because the participants with this infection were more likely to experience a compromised immune response and a higher incidence of chronic infection, than other participants in this study. Preliminary findings suggest that further research to measure the effectiveness of CM treatment on chronic infection is warranted, particularly considering the continuing decline in the effectiveness of antibiotics and the increased risk of viral pandemics spreading globally. Men with the testicular 'super bug' might be considered of particular risk from pandemics if their general immunity is also compromised. This specific profile of sub fertile men is another good example of the potential usefulness of a semen analysis report, in assisting identification of men at risk, who under normal circumstances may go undiagnosed.

Another health problem observed during the study, considered more significant twenty years ago than at present, were fungal and dermatitis infections located around the scrotum and the groin. More than 34 percent of participants (Table 5.11) complained of these specific infections and several authors during the 1980's believed this problem contributed to male sub fertility, due to the associated skin inflammation increasing scrotal temperature (Hargreaves, 1988b). Interestingly, animal studies have revealed that scrotal heat drastically increases the number of sperm cells with malformation (Franken and Kruger, 2004c), however a thorough search of recent journal articles found little evidence of interest in identifying scrotal skin diseases as a risk for male sub fertility. The present study felt that chronic scrotal skin infections might contribute to a male factor however the significance of these infections remains inconclusive because there has been no large scale observational study on the subject. Further research to assess the effectiveness of CM treatment on scrotal skin diseases might add some insight into the discussion and CM research in this area is highly recommended. A large scale observational study investigating the incidence and significance of scrotal skin infections is also highly recommended.

6.2.4 Chinese medicine discussion on kidney yin and yang vacuity leading to male sub fertility

Consistently in CM discussion on the causes of male sub fertility, an emphasis is placed on supplementing kidney yin and yang. The patterns of damp heat and liver qi stagnation are also to a lesser degree cited as contributing factors that need to be addressed. Despite these theories, it was found that other patterns dominated the treatment strategy provided in this study.

While the CM theory on the cause of disease, outlined in Chapter two is widely known, the formulas and strategies previously published on male sub fertility have not shown obvious flexibility or investigative analysis in supporting this theory. For example, the hot damp climate in Sydney, may well have resulted in the higher and perhaps more significant incidence of damp heat diseases in the form of chronic sinusitis, chronic chest infections and scrotal fungal infections observed in this study. No doubt, the local climate, diet and lifestyle of sub fertile men will influence the predominance of certain factors however a discussion on these influences has in the past not taken precedence in CM journal articles on male sub fertility.

Chronic *wei qi* vacuity was also considered more problematic than kidney yin vacuity and yang vacuity patterns among participants in this study. As a result, kidney yin and yang supplements were for the most part not considered appropriate, instead more complex and flexible treatment protocols were adopted, involving clearing damp heat, supplementing *wei qi* and then supplementing kidney yin and yang if time allowed.

6.2.5 Referral practices

The mean time for attempting conception amongst participants was 4.3 years. Despite this long duration of sub fertility, Table 5.7 shows that only two (7.1 %) participants were referred to an urologist for further investigation before joining the trial and no participants were specifically screened for risk of testicular cancer. Thirteen participants in the study were considered at risk of varicocele and testicular cancer. Once the participants were informed of their risk, there was a very high compliance rate, with 12

out of the 13 participants referred to an urologist, consenting. Of this group, seven (58%) participants were found to have a varicocele. None of the referred participants had early or late stage testicular cancer, though they were advised to continue monitoring their risk with an ultrasound of the testes every two years, as recommended by specialists in Europe. (McLauchlan and De Krester, 2003).

As shown, there were a very high percentage of men who were not screened (92.9 %) before the study and these findings suggest that referral practices for these men at risk were very poor. Why medical practitioners and medical staff at IVF centres do not commonly refer sub fertile men for further investigation remains unknown.

This study suggests that the problems related to poor referral practices in Sydney and the inadequate laboratory standards for semen analysis as mentioned in Chapter two, have wide ranging implications that need to be addressed. In the interest of achieving 'best practice' for the public and creating future research opportunities of a high standard, it is recommended that an independent semen analysis laboratory that focuses on maintaining the highest standard of 'strict criteria' semen analysis protocol, as well as following appropriate referral guidelines for men at risk needs to be funded and established as a priority.

An independent laboratory might ideally suit a University research facility. Analysing semen samples generates income and projects of this sort may be valuable to a university because they are inexpensive to establish. Many universities already have the facilities required to analyse semen samples in microbiology and forensic departments. An opportunity for further research, in particular collaborative projects across the University departments would be feasible once a laboratory was established. For example, conducting a large scale observational study profiling sub fertile participants who use the lab facility on a university campus would be very useful in answering some of the important issues raised in this study, such as the actual incidence of varicocele, scrotal skin infections and chronic low grade testicular infection.

Collaborative projects might also include research with a forensic department using hair analysis to test the correlation between a semen analysis profile and the CM patterns identified in the current study that suggest heavy metal poisoning. Determining the accuracy of identifying men at risk and then measuring the effectiveness of treatment would be very useful scientific research.

Further work in observing DNA fragmentation and whether or not appropriate therapy such as CM can reverse DNA damage as suggested recently by Pei et al (2005) after acupuncture, would also be a valuable project with wide ranging implications (Pei et al, 2005).

6.2.6 Genetics versus environmental causes

It was not within the scope of this study to investigate whether genetic factors played a role in contributing to the sub fertility of participants. In the randomised treatment group, genetic factors did not appear to play a role due to the improvements in morphology seen in a very short period of time. In this context, because of the predominant influence of environmental factors, it has been found in some studies that blue collar workers are twice as likely to be sub fertile than their white collar counterparts (Townsend, 2004). To date, biomedicine has not developed a screening program to adequately resolve disputes regarding if a person's health complaint is related to exposure in the work place or not. While these circumstances exist, genetic origins are frequently cited as the cause of semen abnormalities, while men at risk go unidentified. This study suggests, that the scientific community may have access to the appropriate tools to identify men at risk and that in the interest of public health and reducing health costs and disease, it is recommended that tools, such as semen analysis and hair analysis gain broader research opportunities to measure the effectiveness of their use as diagnostic tools investigating heavy metal contamination and identifying men at risk.

Chapter VII: Conclusion

7.1 Future Directions

The results of this very small pilot study are encouraging and suggest that acupuncture and Chinese herbal medicine treatments, may statistically and clinically improve moderately sub fertile semen parameters. This has been the first randomised clinical trial conducted on male sub fertility in Chinese medicine. This is important, because in the treatment of male sub fertility in biomedicine only 16 percent of treatment recommendations are at present based on the results of randomised controlled clinical trials (Irvine, 2004).

It is recommended that the present study be conducted on a larger scale as a double-blind placebo-controlled randomised clinical trial. Due to the high incidence of varicocele, further evidence of the effectiveness of acupuncture post varicocelectomy is also recommended in the form of a randomised double-blind placebo-controlled clinical trial on sub fertile men immediately post varicocelectomy.

Appendix 1 Glossary

Azoospermia: Absence of sperm in the ejaculate.

Blastocyst: Five day old embryo, cultivated Invitro.

Cryptorchidism : When one or both of the testes do not descend.

Hypospadias: A developmental abnormality of the positioning of the urethra in the male.

ICSI: Intra Cytoplasmic sperm injection. 'Healthy' looking sperm are selected to be injected into an egg for the purpose of fertilisation. This technique is used when a man's semen sample has less than 4% normal forms. In this instance there is a concern that the sperm may not otherwise successfully fertilise the eggs in a test tube.

In-vitro fertilisation clinics (IVF clinics): A centre that specialises in offering fertility support services such as Invitro fertilisation; ICSI; and semen analysis.

In-vitro fertilisation (IVF): Fertilisation of an egg by a sperm in a test tube.

Sperm abnormality/morphology: Refers to the shape of the sperm head, neck and tail. High sperm morphology or abnormality refers to high numbers of misshapen sperm.

Spermatogenesis: The entire developmental and maturing process of the sperm.

Testosterone: Produced by the Leydig cells in the testes in response to the pituitary gland releasing LH (luteinising hormone). Testosterone is a male sex hormone or androgen and stimulates the male reproductive glands and encourages muscle, bone and hair growth.

Varicocele: A varicose vein of the testes. It is thought that the vein allows more blood flow in the testes and as a result leads to an increase in temperature and this is thought to reduce count and normal forms. This is a gradual process and as such a man may have a varicocele without seeing significant problems initially.

Varicocelectomy: Surgery on the varicose vein of the testes. There are two procedures offered, ligation and embolisation. Both focus on stopping blood flow through the vein in order to lower the temperature of the testes and improve count and morphology.

Vas Deferens: The tubes that carry the sperm from the testes to the urethra.

Appendix 2 Semen Analysis Form

SEMEN ANALYSIS FORM
(Kindly reproduced with permission from James Leong, Seminologist, Sussex Fertility Services)

Surname…….First Name……Doctor……...
DOB…….
Days of Abstinence…….

Time Date of collection	**am/pm**	**/ /**	**Specimen Complete**	**YES/ NO**	**Time Date received**	**am/pm**	**/ /**
Date of Previous Emission / /				**Method of Collection**			

PARAMETER	OBSERVATION AT HOURS	NORMAL RANGE
Liquefaction		**Complete in 5-20 minutes**
Colour		**Translucent - Yellowish White**
Viscosity		**- to +**
Volume (ml)		**2-6ml**
pH		**7.2 - 8.4**
Sperm Density ($x10^6$)		**$\geq$ 20 x 10^6/ml**
Total Sperm Count ($x10^6$)		**$\geq$ 40 x 10^6**
Motility (%)		**> 50% good**
Motility Index		**$\geq$ 150**
Vitality Test (%)		**> 75% viable**
Agglutination/Aggregation		**- to +**
Fructose Test (if Azoospermia)		**Positive**
Live Sperm morphology %		**$\geq$ 30% normal**

ABNORMAL SPERM MORPHOLOGY				OTHER ABNORMAL CELLS				
Head	**Neck/ Mid-piece**	**Cytoplasmic Droplet**	**Tall**	**Immature Germ Cells**	**Pus Cells**	**Red Blood Cells**	**Seminal Debris**	**Epithelial Cells**

SEMINAL SPERM ANTIBODIES (IMMUNOBEAD TEST)

Appendix 3 Media release

Monday 24 May 2004
Treating male infertility: Chinese medicine put to the test

Traditional Chinese Medicine (TCM) researcher Jann Mehmet knows the effectiveness of acupuncture and Chinese herbal medicine in treating male infertility. Now she is putting her observations to the test in a formal clinical trial of the benefits of TCM, the first time such a study has been conducted in Australia – or overseas.

The randomised clinical trial on male sub fertility is being conducted as part of Ms Mehmet's Master of Science research degree at the University of Technology, Sydney. She is now looking for participants to join the study which will commence on 3 July.

Ms Mehmet, who is Director of the Rozelle Acupuncture and Chinese Medicine Centre, said that in 16 years of clinical practice she has seen excellent results treating men with fertility problems, results backed up by studies in IVF clinics overseas. However, scientific validation was needed to encourage greater acceptance of the benefits of complementary medicine.

"In 1997 after nearly 10 years work with women experiencing infertility I decided to research male infertility in detail," she said. "I found that on many occasions, in as little as six weeks after acupuncture and Chinese herbal medicine, significant improvements in semen parameters could be verified by a semen analysis."

“When a couple experience infertility, there is at least a 50% chance that a male factor is the cause. Despite this fact most of the people who come to me for treatment are women. Of these women only 5 % of male partners are interested in seeking further *medical* investigation or Chinese medicine treatment. This is a great pity when we are seeing such good results with the men we are treating.”

"A 2003 retrospective analysis on 9,000 treatments I gave over three years showed that 45 per cent of sub fertile couples presenting at my clinic had been medically diagnosed with an 'unknown' cause of infertility. My further assessment found that 50 per cent of this group experienced sub fertility as a result of a male factor that had been poorly diagnosed and not thoroughly investigated."

"Couples who have trouble conceiving typically look to IVF programs for help, sometimes without a thorough investigation of the man. IVF programs bypass male fertility problems."

"There's more to this than fertility however, sperm analysis is a good way of showing a man's general health. High levels of abnormal sperm are often an indicator and warning sign of general health problems, including environmental poisoning. Men who have a high level of abnormal sperm are at a greater risk of developing testicular cancer."

"The clinical trial will evaluate whether acupuncture and Chinese herbal medicine can improve sperm count and reduce sperm abnormalities, determining what may be reversible sperm damage and provide an opportunity for men to understand the causes of their sub fertility."

The trial will use World Health Organisation semen analysis criteria to test the semen samples before and after treatment.

Forty men with fertility problems will be selected for the study, with 20 randomly selected into a control group and 20 into an experimental group. The experimental group will undergo free TCM treatment over eight weeks. The control group will receive free treatment after a eight-week wait period.

Semen samples from both groups will be provided before the trial and at the end of the eight weeks. The source group of samples will not be identified to the analyst.

"Treatments will involve acupuncture and Chinese herbal medicine, dietary advice and lifestyle recommendations tailored to individual needs," Ms Mehmet said. "The aim will be to strongly improve all sub fertile semen parameters, but there will be other potential benefits for general health, reduced stress and increased libido."

For more information on the trial see: http://www.thehealthcentre.com.au/

Further Information:

Jann Mehmet, Ph (02) 9818 8517 or 0419 409 816

Issued by:

Terry Clinton, UTS Media Office,
Ph (02) 9514 1623 or 0419 293 261

Appendix 4 Web page advertising the clinical trial

Clinical Trials

Jann Mehmet will be conducting clinical trials at the Rozelle Acupuncture and Chinese Medicine Centre from July 2004. Recruitment for the Clinical trial will be during May and June 2004.

The Clinical trial has been approved by the HREC (Human Research and Ethics Committee) at The University of Technology Sydney. Approval number UTS HREC 2004-029A. Jann Mehmet will publish the results of the study as a requirement of her Master in Science (by thesis) at UTS.

The proposed study

"Does Traditional Chinese Medicine improve semen morphology, motility and count in sub fertile men? A pragmatic randomised clinical trial."

Main objective and significance

- To show the benefits of Traditional Chinese Medicine (TCM) treatments in improving sperm quality in sub fertile men by way of a valid scientific study.
- The research will initiate a debate on what is reversible sperm damage and to what extent male sub fertility can be reversed by TCM treatments. The public will have guidelines for when TCM can be applied successfully.

For Further information click on:

- **Pdf Information for Participants**
- **Pdf Traditional Chinese Medicine Treatment of Male Sub Fertility.**

If after reading the paper, '*Information for Participants*,' you are satisfied with the required selection criteria and are interested in participating in the clinical trial please request a Consent Form and it will be sent to you.

You can request the Consent form by submitting your name and email address in the box provided below.

In keeping with the strict ethical requirements set by the HREC, please be assured that all information you provide will be confidential. All emails will be read by Jann Mehmet only.

ct@thehealthcentre.com.au

Given Name Surname Email address

Appendix 5 Consent form

University of Technology, Sydney

Consent Form

I _____ (*participant's name*) agree to participate in the research project; 'Does Traditional Chinese Medicine improve semen count, motility and morphology in sub fertile men? A pragmatic randomised clinical trial'. (UTS HREC 2004-029A).

I understand that this project is being conducted by Jann Mehmet who is an accredited Chinese Medicine practitioner and Student with the Faculty of Science of UTS College of Traditional Chinese Medicine. The research will contribute towards Jann Mehmet obtaining a Master in Science Degree (by Thesis).

Jann Mehmet
Rozelle Acupuncture and Chinese Medicine Centre
3/698 Darling Street
Rozelle 2039
Ph: 9818 8517
Email: ct@thehealthcentre.com.au

Chris Zaslawski
Supervisor
UTS College of Traditional Chinese Medicine
Faculty of Science
645 Harris Street, Ultimo
Ph: 9514 7856
Email: Chris.Zaslawski@uts.edu.au

I understand that the aim of this study is to evaluate whether Acupuncture and Chinese Herbal Medicine can improve specific sub fertile sperm parameters compared to no treatment at all.

I understand that my participation in this research will involve:

1. Being randomly selected by a computer program and placed into either the control or experimental group.
2. Being available for the time of the clinical trial which starts on the 17th July 2004 and finishes on the 2nd October 2004. I understand that my participation will be for 8 weeks within that time frame.

If placed in the experimental group I need to:

1. Present to the Rozelle Acupuncture and Chinese Medicine Centre for 8 consecutive acupuncture treatments during the time of the clinical trial. I am aware of the inconvenience to me in time as a

result of my commitment to the trial over a continuous 8-week period. I understand that the first treatment will take 1-½ hours of my time and subsequent visits will take around an hour; however I agree to make myself available over that period of time.

2. Take Chinese Herbal remedies if recommended and consider dietary changes prescribed during the course of the 8 week treatment. I agree to accept these suggestions and take the remedies to the best of my ability.
3. Receive a Traditional Chinese Medicine (TCM) treatment from Jann Mehmet using pre sterilised and disposable acupuncture needles registered with the Therapeutic Goods Administration (TGA). I also appreciate that the Chinese Herbal Remedies prescribed are registered with the TGA.
4. Provide two semen samples to be analysed by James Leong at Sussex Fertility Services. One sample is to be provided just before the treatments begin and the final sample is to be provided 8 weeks later which is also when my participation in the clinical trial finishes.
5. Abstain from ejaculating 3-5 days before I give the semen samples on both occasions. I am aware of the importance of ensuring consistency in the testing of the semen samples to be compared before and at the end of the 8 week treatment period.
6. Be aware of the possible minimal risk of mild discomfort or occasional bruising at the site of needle insertion and the rare likelihood of possible loose stools or diarrhoea from the Chinese herbal preparation.
7. Provide personal and confidential details to be documented on a case history questionnaire for the benefit of the study and future research directions in treating male sub fertility. I understand that my name will not be on the form but a coded number will identify me to the researcher only. I accept that this information will be kept private and confidential and access is only granted to the principal researchers who are responsible for storing the files safely. I am informed that research documents must be kept for a minimum of 5 years after the clinical trail is completed and after this time they will be destroyed.
8. I am aware that I can maintain contact with Jann Mehmet during the weekly consultation sessions conducted at the Rozelle Acupuncture and Chinese Medicine Centre over the course of the 8-week clinical trial if I have any concerns about the research. I also understand that I am free to withdraw my participation from this research project at any time I wish and without giving a reason. I understand that the withdrawal from the research will not prejudice my future care if I choose at a later date to consult with the researcher in a professional capacity.

If placed in the control group I need to:

1. Wait 8 weeks before I can receive, if I choose, 8 free acupuncture treatments and 8 weeks of free Chinese herbal remedies from Jann Mehmet at the Rozelle Acupuncture and Chinese Medicine Centre. I am aware that I can receive these treatments at a time that is convenient to me based on appointment availability.

2. Provide 2 semen samples to be analysed by James Leong at Sussex Fertility Services. The 1st to be provided and analysed at the commencement of the clinical trial and the 2nd sample to be provided 8 weeks later when my participation in the clinical trial finishes. I am aware that I will need to make myself available for designated specific times as it will be important to test the effects of the experimental group being treated by herbs and acupuncture compared to no treatment at all over a specific 8 week period.
3. Abstain from ejaculating 3-5 days before I provide the semen samples on both occasions. I am aware of the importance of ensuring consistency in the testing of the semen samples to be compared before and at the end of the 8 week period.
4. During the wait time make no significant changes to my lifestyle and diet or alter my vitamin supplementation.
5. Understand that I am free to withdraw my participation from this research project at any time I wish and without giving a reason. I understand that the withdrawal from the research will not prejudice my future care if I choose at a later date to consult with the researcher in a professional capacity. I know that I may contact Jann Mehmet or her supervisor on the above email or phone number if I have any questions concerning the clinical trial.

I agree that the research data gathered from this project may be published in a form that does not identify me in any way.

_____ ___/___/___

Signed by

_____ ___/___/___

Witnessed by

NOTE:

The University of Technology, Sydney Human Research Ethics Committee, has approved this study. If you have any complaints or reservations about any aspect of your participation in this research, which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer, Ms Louise Abrams (ph: 02 – 9514 9615, Louise.Abrams@uts.edu.au), and quote the UTS HREC reference number. Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

Appendix 6 Participant Information form

University of Technology, Sydney

Male Sub Fertility Clinical Trial 2004

Thank you very much for your interest in this clinical trial which has been approved by the HREC (Human Research and Ethics Committee) at UTS (University of Technology). Approval number: UTS HREC 2004-029A. The results of this Clinical Trial will be published as a requirement of my Master in Science (by thesis) at UTS.

Details of the clinical trial are explained in the attached sheet. I have also elaborated on the selection criteria.

If after reading this paper, 'Information for Participants' you are satisfied with the required selection criteria and are interested in participating in the clinical trial please request a Consent Form and I will send it to you.

You can request the Consent form by telephoning my office on:

(02) 9818 8517

Tuesday – Friday
10 am- 5pm

Saturday
10 am – 3pm

In keeping with the strict ethical requirements set by the HREC, please be assured that all information you provide will be confidential. All correspondence will be read by me only.

With thanks,

Jann Mehmet
Director
Rozelle Acupuncture and Chinese Medicine Centre
Acupuncturist and Herbalist of Chinese Medicine
www.thehealthcentre.com.au
ct@thehealthcentre.com.au

Information for Participants

University of Technology, Sydney

Proposed Clinical Trial

"*Does Traditional Chinese Medicine (TCM) improve sperm count, morphology and motility in sub fertile males? A pragmatic randomised clinical trial."*

Purpose of the study

Some practitioners in the Chinese medicine community have been specialising in male sub fertility and gaining very good results. To be able to make any substantial claims about our results we need to conduct a scientifically valid clinical trial.

Benefits to you

All participants in the study will receive free acupuncture and Chinese herbal medicines that are specifically designed to treat male sub fertility. By improving sub fertile semen parameters the treatments aim to assist a couple in conceiving naturally.
Other benefits may include:

- Increased libido
- Enhanced energy levels and general sense of well being
- Improved health as a result of diet and lifestyle recommendations
- General health needs being investigated
- Advice on sustaining personal health and preventing illness
- Reduction in stress levels
- Improvement in digestive system, muscular skeletal, cardio vascular and immune systems.

Why we need volunteers

In order to test scientifically the benefits of Chinese medicine in treating male sub fertility a group of volunteers suiting selection criteria are needed. The selection criteria are set out at the end of this document.

How does the trial work?

To measure the effects of having a Chinese medicine treatment, a computer program randomly divides the volunteers into the **Treatment group** and the **Waitlist group.** The researchers and the volunteers have no say in who goes into either group. This will minimise bias.

What happens if you are placed in the Treatment group?

The treatment group will receive the acupuncture and Chinese medicine treatment during the study. The effectiveness of the acupuncture and Chinese medicine treatment is measured by comparing 2 semen analysis results. The first is taken just before the treatment begins and the second 8 weeks later, just after treatment ends.

What happens if you are placed in the Waitlist group?

The volunteers in the Waitlist group will need to wait 8 weeks before they are offered free acupuncture and Chinese medicine treatments for 8 weeks. This is to thank you for you participation in the study.

The Waitlist group will need to have 2 semen samples analysed. The importance of the Waitlist group in the study is to measure their semen samples before the clinical trial starts and 8 weeks later. Comparing the semen samples of the Waitlist and Treatment groups at the start and end of the study will determine whether it is beneficial to have a Chinese medicine treatment or no treatment at all.

What are the time commitments?

Time commitment is the most difficult question about this trial. It is unfortunately difficult to be flexible with this arrangement when we are measuring specific parameters in a scientifically valid way.

If you are selected into the **Treatment group** you need to be able to participate in 8 consecutive treatments at the start of the clinical trial. You must also be available to provide semen samples on one of the Saturdays designated below before and after your 8 weeks of treatments. The first consultation will be offered on the day you provide the semen sample or the following day which will be a Sunday. The next 7 treatments will be offered on a Sunday or if more suitable to you during a working week day. We will mostly only need an hour of your time each week for the treatment.

If you are selected into the **Waitlist group** you need to be able to provide a semen sample on one of the Saturdays designated below as your starting date and the Saturday 8 weeks after the starting date.

When will the trial start?

Ideally you should be aware of the dates you may need to make yourself available for treatments and providing semen samples. This will allow you to know whether or not you would like to participate in the study. If you have organised to go on holidays during the trial, then you will not be able to participate.

Recruitment for the clinical trial will be conducted during May, June and July 2004**.** Consent forms need to be signed and delivered by Friday 16th July 2004. Acceptance of consent forms after this date will depend on numbers.

The Clinical trial will commence on Saturday 3rd July 2004 and finish Saturday 2nd October 2004. Your participation will be for 8 weeks only between these dates.

Volunteers in both the Waitlist and Treatment groups can start on any one of the 4 Saturdays listed below once consent forms are received and computer selection is organised. As a result your participation in the study will also finish 8 weeks later on the date given.

Start day 1	First semen sample tested	Saturday 17th July
	Second semen sample tested 8 weeks later	Saturday 11th September
Start day 2	First semen sample tested	Saturday 24th July
	Second semen sample tested 8 weeks later	Saturday 18th September
Start day 3	First semen sample tested	Saturday 31st July
	Second semen sample tested	Saturday 25th September
Start day 4	First semen sample tested	Saturday 7th August
	Second semen sample tested	Saturday 2nd October

Your participation in the trial will start with a semen sample being analysed by James Leong (Seminologist and Fertility Scientist) at Sussex Fertility Services Sydney City, and finish 8 weeks later with a second semen sample analysed by James Leong.

What are the Selection Criteria?

Volunteers will be chosen based on semen parameters below normal levels as specified by WHO (World Health Organisation 1999). Semen parameters below normal suggest levels that may predispose men to sub fertility.

You only need to have one of these parameters below normal to be considered sub fertile and eligible to participate in the study. The levels are:

a) Semen counts less than 20 million per ml collected;
b) Abnormal sperm morphology less than 15 percent;
c) Motility less than 30 percent.
Selection criteria will also exclude men:

- Whose semen morphology is less than 5 percent normal. This is to exclude from the study men whose sub fertility is caused by physical obstruction such as varicocele (varicose veins of the testes). We will be interested in testing this group in a larger study at a later date.
- Whose count is less than 3 million per ml.
- Whose sperm motility is less than 10 percent.
- Who have tested positive for sperm antibodies.
- Who live more than 1 ½ hours away from the lab that will analyse semen samples. The semen samples will be collected at the volunteer's home in a sterilised container and transported to the laboratory within 1 ½ hours. This is a quality control measure to ensure sperm tested are alive and viable. The lab is based in the CBD.
- Who cannot be available on any of the starting dates listed above.
- Who, if selected into the treatment group, cannot be available for 8 consecutive weeks. Four potential starting dates are listed above and the final date allocated will be given a week before participation in the trial commences.

The other selection criteria are:

- Participants must have a previous semen analysis consistent with the eligibility criteria taken no longer than 12 months prior to commencement of recruitment.
- Volunteers need to be between 18 and 60 years of age.
- Volunteers need to be available to provide semen samples on a Saturday as this is the only day that the lab is open. The Saturdays booked for the samples to be collected are listed above.

- Participants need to abstain from ejaculation for 3-5 days before semen samples are given. This is important for both semen samples. Variation will affect the results of the clinical trial; therefore if a participant abstains for 5 days for the first sample, he will also need to abstain for 5 days for the final sample 8 weeks later.
- Treatment of the experimental group will be on Saturday and Sunday throughout the time period of the clinical trial.

Appendix 7 Recruitment brochure

University of Technology, Sydney

Male Sub Fertility Clinical Trial 2004

Participants needed.

Proposed Clinical Trial

"*Does Traditional Chinese Medicine (TCM) improve sperm count, morphology and motility in sub fertile males? A pragmatic randomised clinical trial."*

Purpose of the study

Some practitioners in the Chinese medicine community have been specialising in male sub fertility and gaining very good results. To be able to make any substantial claims about our results we need to conduct a scientifically based clinical trial.

Benefits to you

All participants in the study will receive free acupuncture and Chinese herbal medicines that are specifically designed to treat male sub fertility by improving sub fertile semen levels.

Who is conducting the Clinical Trial?

Jann Mehmet will publish the results of the clinical trial as a requirement of her Master in Science (by thesis) at the University of Technology (UTS).

For More Details go to: www.thehealthcentre.com.au and '*Clinical Trials'*.

Appendix 8 Letter to Urologists

University of Technology, Sydney

Jann Mehmet
Research Student University of Technology
Director and Senior Acupuncturist and Herbalist in Chinese Medicine
Rozelle Acupuncture and Chinese Medicine Centre
Telephone: 02 9818 8517

Dear Doctor _____,

I will be conducting a clinical trial on male sub fertility from July 2004. Recruitment for the Clinical trial will be during May, June and July 2004.The Clinical trial has been approved by the HREC (Human Research and Ethics Committee) at The University of Technology Sydney. Approval number UTS HREC 2004-029A. I will publish the results of the study as a requirement of my Master in Science (by thesis) at the University of Technology. The proposed study is, "Does Traditional Chinese Medicine improve semen morphology, motility and count in sub fertile men? A pragmatic randomised clinical trial."

I have enclosed an example of a brochure. Please contact me if you are happy to support this trial by having this brochure left in your waiting room. I have 16 years clinical experience and specialised in this field since 1997. If you would like more information please call, or visit our web site. www.thehealthcentre.com.au
Regards,

Jann Mehmet

Appendix 9 Medical History questionnaire

Case History Form

Date: _____ ID Number:_____

Please circle relevant age group:

18-35 35-45 45-60 D.O.B: _____

1. History

a) List major illnesses and your age when diagnosed

b) List any operations and your age at the time

c) List any major continuing health problems

d) List any minor health concerns

e) List any medication and length of time you have been taking them

_____
_____
_____
_____

f) List any vitamins you are taking and length of time you have been taking them

_____
_____
_____

g) Have you been exposed in the past to toxic chemicals or heavy metals? Please explain

_____
_____
_____
_____

For the following, please circle the appropriate answer

2. Do you smoke? **Yes No**

3. Do you smoke marijuana? **Yes No**

If yes, how often per day? _____
and/or per week? _____
and/or per month? _____
and/or per year? _____

4.

a. Describe breakfast ie. what type of foods do you frequently eat

_____
_____

b. Describe lunch ie. what type of foods do you frequently eat

_____
_____

c. Describe dinner ie. what type of foods do you frequently eat

_____
_____

d. Do you skip meals? Please explain

_____
_____

e. What time do you eat dinner?

_____

_____

f. Do you have any allergies, food intolerances or foods you know make you sick? If yes please explain

_____

_____

5. DIGESTION

Please circle the appropriate answer and elaborate where necessary

a. Do you experience reflux/heartburn? **Yes** **No**

_____

_____

b. Do you suffer flatulence? **Yes** **No**

_____

_____

c. Do you suffer burping? **Yes** **No**

_____

_____

d. Do you experience abdominal pain? **Yes** **No**

_____

_____

e. Do you experience constipation? **Yes** **No**

_____

_____

f. Do you experience loose stools? **Yes** **No**

If yes how often per day? _____
per week? _____
per month? _____

Are your stools soft, broken or unformed? _____

g. Do you experience diarrhoea (are your stools watery)? **Yes** **No**

If yes how many times per day? _____
per week? _____

per month? _____

h. Do you have blood or mucus in your stools? **Yes** **No**

If yes how often per day? _____
per week? _____
per month? _____

i. Do you experience abdominal bloating? **Yes** **No**

Please circle the appropriate answer and elaborate where necessary

6a. Do you experience night sweats? **Yes** **No**

If yes how often per day? _____
per week? _____
per month? _____

b. Do you experience hot flushing? **Yes** **No**

If yes how often per day? _____
per week? _____
per month? _____

Please comment: _____

c. Do you get hot hands or feet? **Yes** **No**

If yes how often per day? _____
per week? _____
per month? _____

Please comment: _____

d. Do you experience ringing in the ears? **Yes** **No**

If yes is it loud? _____
or low? _____

e. Do you experience night time urination? **Yes** **No**

If yes how often: _____

f. Do you prefer hot or cold weather?_____

g. Do you get fluid retention? **Yes** **No**

If yes where and when: _____

h. Do you suffer anxiety? **Yes** **No**

h. Do you experience restless sleep? **Yes** **No**

If yes describe: _____

j. How many hours of sleep do you have per night? _____

k. Urinary frequency during the day? _____

Please circle the appropriate answer and elaborate where necessary

7. Do you experience any of the following:

a. Irritability? **Yes** **No**

If yes, would you describe it as mild, moderate or extreme?

_____

b. High Blood Pressure? **Yes** **No**

If yes, what is your reading? _____

c. Low Blood Pressure? **Yes** **No**

If yes what is your reading? _____

d. Hot itchy eyes? **Yes** **No**

e. Back or leg pain? **Yes** **No**

If yes please explain_____

f. Leg cramps? **Yes** **No**

g. Dizziness? **Yes** **No**

Please circle the appropriate answer and elaborate where necessary

8. Do you experience any of the following:

a. Frequent infections? **Yes** **No**

If yes please describe _____

b. Swollen glands? **Yes** **No**

If yes please explain _____

c. Hay-fever, sneezing, watery eyes, runny nose, nasal drip down the back of the throat? **Yes** **No**

If yes describe which symptoms mentioned above?

_____

d. Wheezing? **Yes** **No**

e. Tight chest? **Yes** **No**

f. Bronchitis/chest infections? **Yes** **No**

Please circle the appropriate answer and elaborate where necessary

9. Do you experience any of the following:

a. Headaches? **Yes** **No**

If yes please describe _____

b. Poor memory or concentration? **Yes** **No**

c. Easily bruised? **Yes** **No**

d. Skin irritations? **Yes** **No**

If yes please describe _____

Please circle the appropriate answer and elaborate where necessary

10. Do you now or in the past experience any of the following:

a. Testicular swelling? **Yes** **No**

b. Sharp pain in the testicles? **Yes** **No**

c. Itchiness of the scrotum, anus or groin area? **Yes** **No**

If yes please explain _____

d. Injury to the groin ie. a hard hit? **Yes** **No**

e. Have you had an ultrasound for a varicocele? **Yes** **No**

OFFICE USE

TONGUE

PULSE

TENDERNESS ON PALPATION

DIAGNOSIS

Toxic Liver
Liver Qi stasis
Damp
Kidney Yin Vacuity
Kid Yang Vacuity
Qi Vacuity
Blood Vacuity
Blood Stasis
Parasites
Other

TREATMENT PROTOCOL

HERBS

ACUPUNCTURE

Appendix 10 Participant feedback form

University of Technology, Sydney

Clinical Trial 2004

I would like to formally thank you for participating in the clinical trial and completing all the requirements. Your contribution has been very valuable.

In an effort to improve organisation and satisfaction in participating in future trials, feed back is useful. I would really value and appreciate any honest feed back that you may have at this time.

Best wishes for the future,

Jann Mehmet.

Participant Feedback Form

1. Communicating and providing information through the internet has been a recruitment initiate of this trial. Please rate your approval by circling the most appropriate answer.

a) Did the web site or initial communication and information via emails assist you in feeling more comfortable about making enquiries about the trial?

Did not use the internet Not Helpful Helpful Very Helpful

b) Was the information on the web site or communication via emails helpful in making you decide to join the trial?

Did not use the internet Not Helpful Helpful Very Helpful

2. Did you find the trial well organised?

No Fair Good Very good

3. Did you have any apprehensions about working with a female practitioner?

   No Some Yes

4. Do you think a male practitioner would be more suitable?

   No Not sure Perhaps Yes

5. Have you received acupuncture before the trial?

   Yes No

6. Have you taken Chinese herbal medicines before the trial?

   Yes No

7. Would you consider having acupuncture or Chinese herbal medicine treatments in the future?

   Yes Not sure No

8. Rate the benefits to your health as a result of your participation in the trial. Please circle.

   None Slight Good V Good Excellent

9. Are you glad that you participated in the trial? Circle.

   Unhappy neutral Pleased Very Pleased

Further comments_____

_____

_____

_____

_____

_____

Thank you

Appendix 11 Treatment given to the experimental group

All Chinese herbal medicines selected and prescribed were single granulated Sun Ten herbs, unless otherwise stated. Five grams (gms) of the mixture was recommended per day with a warm tea.

Participant J101 Treatment one

Chai hu 3gm, *bai shao* 5gm, *sheng jiang* 2gm, *ban xia* 2.5gm, *qing hao* 4gm, *gua lou gen* 5gm, *gan cao* 2gm, *lian qiao* 4.1gm, *mai men dong* 3.5gm, *huang lian* 3.5gm, *mu xiang* 3.5gm.

LR3, LI4, CY17, CV4, CV6, ST25, KI16. After 20 minutes change to LU 7 for five minutes.

Treatment two

Chai hu 3gm, *bai shao* 9gm, *qing hao* 3gm, *tu si zi* 6gm, *zhe ke* 3.2gm, *gua lou gen* 2.5gm, *huang lian* 3gm, *mu xiang* 3gm, *gan cao* 1.5gm.

LR3, LI4, CV16, CV4, CV6, ST25. After 20 minutes change acupuncture points to LU7 for five minutes.

Treatment three

Chai hu 3gm, *bai shao* 9gm, *tu si zi* 6 gm, *zhe ke* 3.2gm, *huang qin* 4gm, *huang lian* 3gm *mu xiang,* 3gm, *gan cao* 2gm.

LR3, LI4, SP6, CV4, CV6, KI16, ST15. After 20 minutes change acupuncture points to LU7

Treatment four

Long gu 5.5gm*, mai men dong tang* 11gm, *xiang sha liu jun zi tang* 7.5gm, *chai hu* 2gm, *bai shao* 4gm, *huang liang* 9gm, *tu si zi* 5gm, *wu wei zi* 4gm, *zhe ke* 2gm.

CV17, CV4, CV6, PC6, LR3, LR8, LU4, KI16, HT7. After 20 minutes change acupuncture points to LU7

Treatment five

Long gu 3gm*, mai men dong tang* 10gm, *xiang sha liu jun zi tang* 10gm, *chai hu* 3gm, *bai shao* 8gm, *huang qi* 3gm, *tu si zi* 5gm, *wu wei zi* 4gm, *zhe ke* 2gm.

LR3, LI4, SP6, CV6, CV4, KI16, ST25, ST30. After 20 minutes change acupuncture points to LU7

Treatment six

Long gu 3gm, *mai men dong tang* 10gm, *xiang sha liu jun zi tang* 10gm, *chai hu* 3gm, *bai shao* 3gm, *huang lian* 3gm, *wu wei zi* 4gm, *zhe ke* 2gm, *mu li* 4gm, *gan cao* 2gm.

PC6, HT7, CV17, CV4, CV6, SP6, ST36, ST25, KI16. After 20 minutes change acupuncture points to LU7

Treatment seven

Long gu 4gm *mai men dong tang* 7gm, *chai hu* 5gm, *bai shao* 10gm, *qing hao* 3gm, *huang lian* 3gm, *wu wei zi* 4gm, *zhe ke* 3.2gm, *tu si zi* 6gm, *suan zuo ren* 5gm, *huang qin* 2gm, *gan cao* 2gm, *hu ma ren* 3gm.

LR3, LR8, LU4, CV17, CV6, CV4, KI16, ST25. After 20 minutes change acupuncture points to LU7

Treatment eight

Long gu 4gm *mai men dong tang* 7gm, *chai hu* 5gm, *bai shao* 10gm, *qing hao* 3gm, *huang qi* 3gm, *wu wei zi* 4gm, *zhe ke* 3.2gm, *tu si zi* 6gm, *suan zuo ren* 5gm*, huang qin* 2gm, *gan cao* 2gm, *hu ma ren* 3gm.

LR3, LR2, LI4, ST25, KI16, CV4, CV6. After 20 minutes change acupuncture points to LU7

Participant J104

Treatment one

Mai men dong tang 19gm, *xiang sha liu jun zi tang* 16gm, *chai hu* 4gm, *bai shao* 3.5gm, *hai piao xiao* 5gm, *long gu* 3gm, *wu wei zi* 3gm, *bu gu zhi* 3gm, *gan cao* 2gm.
Men's treasure pills 30 pills per day.

LR3, LI4, ST25, KI16, CV4 CV6 ST30.

Treatment two

Mai men dong tang 19gm, *xiang sha liu jun zi tang* 16gm, *chai hu* 4gm, *bai shao* 3.5gm, *hai piao xiao* 5gm, *long gu* 3gm, *wu wei zi* 3gm, *bu gu zhi* 3gm, *gan cao* 2gm. Men's treasure pills 30 pills per day.

PC6, HT7, ST25, ST36, SP6, CV17, CV4, CV6, KI16.

Treatment three

Mai men dong tang 19gm, *xiang sha liu jun zi tang* 16gm, *chai hu* 4gm, *bai shao* 3.5gm, *hai piao xiao* 5gm, *long gu* 3gm, *wu wei zi* 3gm, *bu gu zhi* 3gm, *gan cao* 2gm. Men's treasure pills 30 pills per day.

LR3, LI4, SP6, ST25, CV4, CV6, GV20, SI3.

Treatment four

Mai men dong tang 19gm, *xiang sha liu jun zi tang* 16gm, *chai hu* 4gm, *bai shao* 3.5gm, *hai piao xiao* 5gm, *long gu* 3gm, *wu wei zi* 3gm, *bu gu zhi* 3gm, *gan cao* 2gm. Men's treasure pills 30 pills per day.

PC6, HT7, LR3, LR2, ST25, ST36, CV4, CV6, KI16

Treatment five

Mai men dong tang 19gm, *xiang sha liu jun zi tang* 16gm, *chai hu* 4gm, *bai shao* 3.5gm, *hai piao xiao* 5gm, *long gu* 3gm, *wu wei zi* 3gm, *bu gu zhi* 3gm, *gan cao* 2gm. Men's treasure pills 30 pills per day.

LR3, LR2, CV17, CV4, CV6, ST25, KI16. Change acupuncture points to LU7, LI4, TE5, ST36, KI4, for ten minutes.

Treatment six

Mai men dong tang 19gm, *xiang sha liu jun zi tang* 16gm, *chai hu* 4gm, *bai shao* 3.5gm, *hai piao xiao* 5gm, *long gu* 3gm, *wu wei zi* 3gm, *bu gu zhi* 3gm, *gan cao* 2gm. Men's treasure pills 30 pills per day.

LR3, LI4, SP6 CV4, CV6, KI16, ST26. Change acupuncture points to LU7, TE5, LU 4, ST36, for 10 minutes.

Treatment seven

Mai men dong tang 19gm, *xiang sha liu jun zi tang* 16gm, *chai hu* 4gm, *bai shao* 3.5gm, *hai piao xiao* 5gm, *long gu* 3gm, *wu wei zi* 3gm, *bu gu zhi* 3gm, *gan cao* 2gm. Men's treasure pills 30 pills per day.

CV17, CV4, CV6, PC6, HT7, ST 36, LR3, SP6, KI 16

Treatment eight

Mai men dong tang 19gm, *xiang sha liu jun zi tang* 16gm, *chai hu* 4gm, *bai shao* 3.5gm, *hai piao xiao* 5gm, *long gu* 3gm, *wu wei zi* 3gm, *bu gu zhi* 3gm, *gan cao* 2gm. Men's treasure pills 30 pills per day.

CV17, CV4, CV6, PC6, HT7, ST 36, LR3, SP6, KI 16

Participant J109 Treatment one

Xiao chai hu tang, 4 pills 2 x per day.

LR3, LI4, ST25, KI16 ST28 CV4 CV6. After 20 minutes change acupuncture points to LU7

Treatment two

Xiao chai hu tang, 4 pills 2 x per day.

LR3, LI4, ST25, KI16 ST28 CV4 CV6. After 20 minutes change acupuncture points to LU7

Treatment three

Xiao chai hu tang, 4 pills 2 x per day.

LR3, LI4, ST25, KI16 ST28 CV4 CV6, for 20 minutes and then change acupuncture points to LU7 and ST36 for 10 minutes.

Treatment four

Xiao chai hu tang, 4 pills 2 x per day.

LR3, LI4, ST25, KI16 ST28 CV4 CV6, for 20 minutes and then change acupuncture points to LU7 and ST36 for 10 minutes.

.

Treatment five

Xiao chai hu tang, 4 pills 2 x per day. Men's treasure pills, 30 pills 2 x per day.

LR3, LI4, ST25, KI16 ST28 CV4 CV6, for 20 minutes and then change acupuncture points to LU7 and ST36 for 10 minutes.

.

Treatment six

Xiao chai hu tang, 4 pills 2 x per day. Men's treasure pills, 30 pills 2 x per day.

LR3, LI4, ST25, KI16 ST28 CV4 CV6, for 20 minutes and then change acupuncture points to LU7 and ST36 for 10 minutes.

Treatment seven

Xiao chai hu tang, 4 pills 2 x per day. Men's treasure pills, 30 pills 2 x per day.

LR3, LI4, ST25, KI16 ST28 CV4 CV6, for 20 minutes and then change acupuncture points to LU7 and ST36 for 10 minutes.

Treatment eight

Xiao chai hu tang, 4 pills 2 x per day. Men's treasure pills, 30 pills 2 x per day.

LR3, LI4, ST25, KI16 ST28 CV4 CV6, for 20 minutes and then change acupuncture points to LU7 and ST36 for 10 minutes.

Participant J110 Treatment one

Men's treasure pills, 30 tablets per day.

LR3, LI4, KI16 CV4, CV6.

Treatment two

Men's treasure pills, 30 tablets per day.

LR3, LI4, KI16 CV4, CV6.

Treatment three

Men's treasure pills, 30 tablets per day.

LR3, LR8, SP6, KI16, LI4, CV4, CV6.

Treatment four

Men's treasure pills, 30 tablets per day.

LR3, LI4, SP6, KI16, LR8, CV4, CV6.

Treatment five

Men's treasure pills, 30 tablets per day.

LR3, LR8, LI4, SP6, KI16, CV4, CV6.

Treatment six

Men's treasure pills, 30 tablets per day.

LR3, LR8, LI4, SP6, KI16, CV4, CV6.

Treatment seven

Sheng di huang 3gm, *he shou wu* 4gm, *ji xue teng* 4gm, *sang ji sheng* 3gm, *suan zao ren* 3g, *nu zhen zi* 4gm, *gou qi zi* 4gm, *zhi mu* 2gm, *huang bai* 2gm, *bai shao* 6gm, *chai hu* 4gm, *fu ling* 3gm, *shan yao* 3gm, *niu xi* 3gm, *gan cao* 2gm.

LR3, SP6, KI16, LI4, CV4, CV6.

Treatment eight

Sheng di huang 3gm, *he shou wu* 4gm, *ji xue teng* 4gm, *sang ji sheng* 3gm, *suan zao ren* 3g, *nu zhen zi* 4gm, *gou qi zi* 4gm, *zhi mu* 2gm, *huang bai* 2gm, *bai shao* 6gm, *chai hu* 4gm, *fu ling* 3gm, *shan yao* 3gm, *niu xi* 3gm, *gan cao* 2gm.

LR3, LI4, KI16, CV4, CV6, LR8, SP6.

Participant J114 Treatment one

Xiao chai hu tang, 4 pills 2 x per day. *Guo luo shi* 8.5gm, *bai he* 7.5gm, *huang lian* 5.5gm, *huang qin* 7gm, *xing ren* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, SP6, LI4, ST25, KI16, CV4, CV6. After 20 minutes change acupuncture points to LU7

Treatment two

Xiao chai hu tang, 4 pills 2 x per day. *Guo luo shi* 8.5gm, *bai he* 7.5gm, *huang lian* 5.5gm, *huang qin* 7gm, *xing ren* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, LI4, SP6, KI16, CV4, CV6. After 20 minutes change acupuncture points to LU7

Treatment three

Xiao chai hu tang, 4 pills 2 x per day. *Guo luo shi* 8.5gm, *bai he* 7.5gm, *huang lian* 5.5gm, *huang qin* 7gm, *xing ren* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, LI4, SP6, CV4, CV6, KI16. After 20 minutes change acupuncture points to LU7

Treatment four

Xiao chai hu tang, 4 pills 2 x per day. Men's treasure pills, 30 pills per day.

LR3, LR8, LI4, KI16, CV4, CV6, GB20, GB 21. After 20 minutes change acupuncture points to LU7

Treatment five

Xiao chai hu tang, 4 pills 2 x per day. Men's treasure pills, 30 pills per day.

LR3, LR2, LI4, ST25, KI16, CV4, CV6. After 20 minutes change acupuncture points to LU7

Treatment six

Xiao chai hu tang, 4 pills 2 x per day. Men's treasure pills, 30 pills per day.

LR3, LR2, LI4, ST25, KI16, CV4, CV6. After 20 minutes change acupuncture points to LU7

Treatment seven

Zhe mu 3.3gm, *huang bai* 3gm, *tian men dong* 3gm, *gou qi zi* 3gm, *shu di huang* 4gm, *he sho wu* 4.2gm, *dang shen* 3.2gm, *mu dan pi* 3.5gm, *shan yao* 3.5gm, *fu ling* 3gm, *nu zhen zi* 2.5gm, *gan cao* 2gm, *shan zhu yu* 3gm.

LR3, LR2, LI4, ST25, KI16, CV4, CV6.

Treatment eight

Zhe mu 3.3gm, *huang bai* 3gm, *tian men dong* 3gm, *gou qi zi* 3gm, *shu di huang* 4gm, *he sho wu* 4.2gm, *dang shen* 3.2gm, *mu dan pi* 3.5gm, *shan yao* 3.5gm, *fu ling* 3gm, *nu zhen zi* 2.5gm, *gan cao* 2gm, *shan zhu yu* 3gm.

LR3, LR2, LI4, ST25, KI16, CV4, CV6.

Participant J127 Treatment one

Xiao chai hu tang, 4 pills 3 x per day. Men's treasure pills, 30 pills 2 x per day.

LR3, LI4, ST25, KI16, CV4, CV6, GB 40, BL62.

Treatment two

Xiao chai hu tang, 4 pills 3 x per day. Men's treasure pills, 30 pills 2 x per day.

LR3, LI4, ST25, KI16, CV4, CV6.

Treatment three

Xiao chai hu tang, 4 pills 3 x per day. Men's treasure pills, 30 pills 2 x per day.

LR3, LI4, CV3, CV4, CV6, KI16, CV17 and local pts for an injured ankle left side, BL60, BL62, GB40.

Treatment four

Xiao chai hu tang, 4 pills 3 x per day. Men's treasure pills, 30 pills 2 x per day.

LR3, LI4, BL23, BL24, BL25, BL26, BL27 and local ah shi painful points around the left ankle.

Treatment five

Zhi mu 3gm, *huang bai* 3.5gm, *tian men dong* 3gm, *gou qi zi* 3.5gm, *tu si zi* 4gm, *he shou wu* 3.5gm, *suan suo ren* 3.5gm, *fu ling* 4gm, *nu zhen zi* 3gm, *gan cao* 2gm.

LR3, LI4, ST25, CV4, CV6, CV3.

Treatment six

Zhi mu 3gm, *huang bai* 3.5gm, *tian men dong* 3gm, *gou qi zi* 3.5gm, *tu si zi* 4gm, *he shou wu* 3.5gm, *suan suo ren* 3.5gm, *fu ling* 4gm, *nu zhen zi* 3gm, *gan cao* 2gm.

LR3, LI4, BL23, BL24, BL26 and local ah shi pts around the iliac.

Treatment seven

Zhi mu 3gm, *huang bai* 3.5gm, *tian men dong* 3gm, *gou qi zi* 7.5gm, shan yao 4gm, *he shou wu* 3.5gm, *fu ling* 4gm, *nu zhen zi* 2.5gm, *dang shen* 5gm, *gan cao* 2gm.

LU7 (left), KI6 (right), SI3, BL23, BL24, BL25, BL26, change acupuncture points after 20 minutes to KI6 (left), LU7 (right), for 10 minutes.

Treatment eight

Zhi mu 3gm, *huang bai* 3.5gm, *tian men dong* 3gm, *gou qi zi* 7.5gm, shan yao 4gm, *he shou wu* 3.5gm, *fu ling* 4gm, *nu zhen zi* 2.5gm, *dang shen* 5gm, *gan cao* 2gm.

LI4, LU7, GB34, ST36, BL24, BL25, BL26

Participant J130 Treatment one

Guo luo shi 8.5gm, *bai he* 7.5gm, *huang lian* 5.5gm, *huang qin* 7gm, *xing ren* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, LI4, ST25, CV4, CV6, KI16, CV17, KI27 and after 20 minutes, change acupuncture points to LI4, LU7.

Treatment two

Guo luo shi 8.5gm, *bai he* 7.5gm, *huang lian* 5.5gm, *huang qin* 7gm, *xing ren* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, LI4, ST25, CV4, CV6, KI16, CV17, KI27 and after 20 minutes, change acupuncture points to LI4, LU7 LU5.

Treatment three

Guo luo shi 8.5gm, *bai he* 7.5gm, *huang lian* 5.5gm, *huang qin* 7gm, *xing ren* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, SP6, LI4, KI16, KI27, ST25, SI17 and after 20 minutes change acupuncture points to LI4, LU7.

Treatment four

Mai men dong 4.5gm, *xuan shen* 4gm, *bai he* 7.5gm, *huang lian* 5.5gm, *huang qin* 7gm, *xing ren* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, LI4, SP6, BL43, GB20, BL14 13 and after 20 minutes change acupuncture points to LI4, LU 7, LU5.

Treatment five

Mai men dong tang 8gm, *xuan shen* 3gm, *bai he* 7.5gm, *huang lian* 5.5gm, *huang qin* 7gm, *xing ren* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, LI4, SP6, BL43, GB20, GB21, BL10 BL18, BL19, BL20, BL21 and after 20 minutes change acupuncture points to LI4, LU7.

Treatment six

Mai men dong tang 8gm, *xuan shen* 3gm, *bai he* 7.5gm, *bai shao* 5.5gm, *huang qin* 7gm, *gou qi zi* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, SP6, CV6, CV4, KI16 and after 20 minutes change acupuncture points to HT7.

Treatment seven

Mai men dong tang 8gm, *xuan shen* 3gm, *bai he* 7.5gm, *bai shao* 5.5gm, *huang qin* 7gm, *gou qi zi* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, SP6, CV6, CV4, KI16 and after 20 minutes change acupuncture points to HT7.

Treatment eight

Mai men dong tang 8gm, *xuan shen* 3gm, *bai he* 7.5gm, *huang lian* 5.5gm, *huang qin* 7gm, *xing ren* 8gm, *shan zha* 4.5gm, *sha ren* 5.5gm, *gan cao* 2gm.

LR3, LI4, CV17, CV4, CV6, ST25, KI16, KI16, SI17 and after 20 minutes change acupuncture points to LU7, ST36.

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