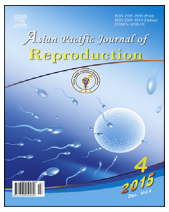
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net



Original research http://dx.doi.org/10.1016/j.apjr.2015.07.004

A single-arm pilot study on effects of acupuncture treatment on semen parameters of subfertile Singaporean men

Lee Mee Ho[1], Shu Li Chui[2], Siew Chen Hum[1], Johnny Qi Ming Foo[3], Stephanie Man Chung Fook-Chong[4], Shaw Fong Chong[3], Man Geng[3], Kim Gek Loh[3], Kin Yee Loh[3], Shaw Ni Lee[1], Su Ling Yu[1], Tze Tein Yong[1*]

[1]*Centre for Assisted Reproduction (CARE), Department of Obstetrics and Gynaecology, Singapore General Hospital, Singapore*

[2]*Acupuncture Services Clinic, Department of Pain Management Centre, Singapore General Hospital, Singapore*

[3]*Singapore Thong Chai Medical Institution, Singapore*

[4]*Health Services Research and Biostatistics, Department of Clinical Research, Singapore General Hospital, Singapore*

ARTICLE INFO

Article history:
Received 13 Jun 2015
Received in revised form 10 Jul 2015
Accepted 16 Jul 2015
Available online 4 Aug 2015

Keywords:
Acupuncture
Male subfertility
Semen parameters
Sperm-hyaluronan binding assay (HBA)
Mature sperm

ABSTRACT

Objective: To evaluate the effect of acupuncture treatment on semen parameters and the proportion of mature and functional sperm as measured by using sperm-hyaluronan binding assay (HBA) in subfertile Singapore men.

Methods: Singaporean male subjects undergoing subfertility investigation were enrolled prospectively in this pilot study. Each subject received acupuncture treatment at fixed acupoints and two acupuncture sessions per week for 10 weeks. A total of five semen samples were obtained from each subject at 5 weekly intervals up to 20 weeks of the study; at Pre-treatment, Treatment 1 (5th week), Treatment 2 (10th week), Post-treatment 1 (15th week) and Post-treatment 2 (20th week). Semen analysis was performed to assess semen parameters and HBA score (percentage of motile sperm bound to hyaluronan) to determine the proportion of mature and functional sperm.

Results: Mean HBA scores improved significantly from 12.0% at Pre-treatment, to 18.4% ($P = 0.03$) at 5th week and was 16.8% ($P = 0.07$) at 10th week of acupuncture treatment. After completion of acupuncture treatment, HBA scores continued to increase significantly to 25.8% ($P < 0.001$) at Post-treatment 1. At Post-treatment 2, HBA scores decreased to 16.3%. Sperm concentration showed similar trend but it was not statistically significant. Other semen parameters remained unchanged throughout the study period.

Conclusions: HBA scores increased significantly during the first 5 weeks of acupuncture treatment (Treatment 1) and 5 weeks after the completion of treatment (Post-treatment 1). Hence acupuncture treatment was effective in increasing the proportion of mature and functional sperm but had no effect on other semen parameters.

1. Introduction

Men with impaired semen parameters account for up to 50% of infertility among subfertile couples. Conventional therapies such as over the counter vitamins and supplements have been used widely, but it may not be effective in improving semen parameters and the true effects on dosing regimen could not be identified [1]. These subfertile men may not be able to get their partners to conceive naturally. The only option of having a baby would be to undergo invasive assisted reproduction treatment.

According to traditional Chinese medicine (TCM), health is achieved by maintaining the body in a 'balance state' and disease is caused by an imbalance in the body. The imbalance causes a blockage to the natural flow of vital energy, known as *qi* along the meridians. Acupuncture is a medical technique unique to TCM, and has been practiced for more than 2 000 years to treat a whole range of illnesses. It involves inserting

*Corresponding author: Dr. Tze Tein Yong, Department of Obstetrics and Gynaecology, The Academia, Level 5, 20 College Road, Singapore 169856, Singapore.
Tel: +65 63214673
Fax: +65 62253464
E-mail: yong.tze.tein@sgh.com.sg

Peer review under responsibility of Hainan Medical College.
Foundation Project: This study was supported by SingHealth-Traditional Chinese Medicine research grant in Singapore (SHF/TCM003/2008).

needles at various acupoints along the body that connect the meridians to unblock the *qi* and restores the body's balance [2]. Male infertility could be due to the 'deficiency of the kidneys' or 'damp heat in the genital system' [3]. The former syndrome is usually implicated as the aetiology to spermatogenic failure and the latter to the inflammation of the genital tract.

Acupuncture has shown a positive effect on sperm concentration and motility as well as increase in testosterone level in infertile men [4]. Other studies have demonstrated that acupuncture treatment improve sperm parameters of men suffering from impaired sperm quality [5–9].

Sperm-hyaluronan binding assay (HBA) is a simple, patented and non-invasive *in-vitro* diagnostic test for evaluating sperm maturity, quality and fertilizing ability. Hyaluronan is a major constituent of the cumulus oophorous matrix surrounding the human oocyte. During the process of fertilisation, hyaluronan plays an important role in the selection of functionally competent sperm. HBA is a useful indicator of completion of normal sperm spermatogenesis, based on increased staining for various maturity markers including creatine kinase, HSPA2 and Pisum sativum lectin. Sperm that show binding to hyaluronan is mature and has the capability to bind to and fertilise an egg. Sperm that shows no binding is unable to fertilise and is likely to have other defects, including chromosomal abnormalities and reduced DNA integrity [10–13].

Acupuncture as a possible treatment option for subfertile men prompted the evaluation of the effect of acupuncture treatment on semen parameters and the proportion of mature and functional sperm for subfertile Asian men living in Singapore.

2. Materials and methods

2.1. Subjects

Male subjects of Asian origin undergoing preliminary subfertility investigation either at the Singapore General Hospital or Singapore Thong Chai Medical Institution, Singapore, were enrolled prospectively in this pilot study between July 2009 and October 2012. Subjects were willing to receive acupuncture treatment and had given their written informed consent prior to the start of acupuncture treatment.

These couples were not able to achieve pregnancy after trying for a period of >1 year. The inclusion criteria were: Male subjects of age between 21 and 55 years old; with initial sperm concentration of >15 million sperm/mL, progressive motility >20% and HBA score of <40%. The exclusion criteria were: Male subjects who were azoospermic or had undergone any surgical procedures related to male infertility within 1 year prior to the enrolment period. The investigation protocol for this study was approved by the SingHealth Centralised Institutional Review Board and was funded by the SingHealth Foundation, Singapore.

2.2. Acupuncture treatment

Acupuncture treatment was performed by one of the two designated acupuncturist who is TCM/Acupuncture trained and certified. One acupuncture treatment cycle consisted of twice weekly sessions over 5 weeks. All subjects were given two treatment cycles as a complete treatment regime and at fixed acupoints, regardless of their TCM diagnosis. A total of 20 acupuncture sessions were performed over a period of 10 weeks and to a maximum of 12 weeks, as some subjects were unable to attend treatment on time.

Twelve subjects had completed the acupuncture treatment while the rest discontinued treatment due to various reasons. All 12 subjects received needling acupuncture, which were similar to the acupoints used by Pei J *et al.*, in their study on idiopathic men [9]. In our study, *Xue Hai* acupoint was omitted. The main acupuncture points used were: *Guan Yuan, Shen Shu* (bilateral), *Ci Liao* (bilateral), *Tai Cong* (bilateral) and *Tai Xi* (bilateral). The secondary points were *Bai Hui, Zhu San Li* (bilateral), *San Yin Jiao* (bilateral) and *Gui Lai* (bilateral). The locations of acupoints were according to the international standardized location of acupoints. Sterile disposable stainless steel needles [(0.25 × 25) mm or (0.30 × 50) mm], (Tian Sie, Medical Appliance Factory, Suzhou, China) were inserted to a depth of 15–25 mm, depending on the region of the body undergoing treatment and according to the accepted rules of acupuncture treatment. To evoke the needle sensation, or *De qi* (often described as variable feeling of soreness, numbness, tingling, warmness, and/or distension around the acupoints), the 'lifting, thrusting and rotating manipulating techniques' were used. When puncturing *Gui Lai*, and *Ci Liao*, the needling sensations were transmitted to the sacral or perineum area and anterior hypo gastric zone.

Electro-acupuncture technique was used (HANS, Singapore) during the acupuncture treatment. Acupuncture needles were inserted at two acupoint pairs; *Gui Lai* and *San Yin Jiao*, and *Ci Liao* and *Tai Xi.* These needles were used as electrodes for passing weak electric current (approximate frequency of 10 mA & intensity of 100 Hz). During each session, 10 needles were inserted on the frontal part of the body and withdrawn after 30 minutes. Six more needles were then inserted at the back of the body while subject was lying in a prone position for 30 minutes. Thus, a total of 16 acupoints were used for every single treatment session.

2.3. Semen collection and analysis

Subjects were required to collect their semen sample by masturbation after a period of sexual abstinence of 3 days. Semen analysis and the HBA test were carried out for all semen samples within 2 h of collection.

Freshly ejaculated semen samples were assessed for semen volume and sperm parameters [progressive motility (grade a + b), *in situ* progression (motility grade c), vitality, and sperm concentration] according to World Health Organization (WHO) 1999 guidelines [14]. An aliquot of the semen was smeared on a glass slide, fixed in 95% ethanol solution and stained using the Hemacolor Red and Blue solutions (Merck, KGa A, Darmstadt, Germany) for the determination of normal sperm morphology, following the Kruger Strict Criteria [15]. A total of 400 sperm were counted for each of the sperm parameters from two aliquots of each sample and the average score was determined. Total motile sperm concentration (TMSC) was calculated by multiplying semen volume, progressive motility and sperm concentration.

A total of 5 semen samples were collected at various time points during the study period. The first sample (Pre-treatment) was obtained on the day before the start of acupuncture treatment. After the submission of the sample, subject started his acupuncture sessions, twice a week for 5 weeks. The second sample (Treatment 1) was obtained after the 10th session and the