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

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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

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has been practiced for more than 2 000 years to treat a whole range of illnesses. It involves inserting 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 etiology 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. This study was approved by the SingHealth Centralized Institutional Review Board, SHS IRB 2009/594/D.

These couples were not able to achieve pregnancy after trying for a period of >1 year. The inclusion criteria were: Male subjects aged between 21 to 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 to 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, warmth, 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. 6 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 hours 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 third sample (Treatment 2) was after the completion of the 20th session. The 4th sample (Post-treatment 1) was obtained 5 to 6 weeks and the 5th sample (Post-treatment 2) was obtained on the 10 to 12 weeks after the completion of 20th acupuncture treatment session.

2.4. Sperm–hyaluronan binding assay (HBA)

HBA was assessed using commercial HBA kit (Biocoat INC, Fort Washington, PA, USA). Briefly, an aliquot of the fresh semen was placed at the centre of the HBA chamber slide and covered with a Cell-Vu grid cover slip. A grid of 100 squares (measuring 0.1 mm x 0.1 mm) is provided on the cover slip.

After incubation period of 15 minutes at room temperature, a total of 400 bound and unbound motile sperms were counted in the same grid squares. Hyaluronan-bound sperm showed no progressive head movement but with rapid beating of their tails, the unbound sperm swam around freely. The HBA score (in percentage) was calculated

by determining the fraction of bound motile sperm divided by the total number of motile sperm (bound + unbound free swimming) x 100.

2.5. Statistical analysis

All statistical evaluation was performed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). Data was presented as mean±SD (standard deviation) and ranges (minimum to maximum). ANOVA with repeated measure, linear trend over time comparisons within subjects was used to analyze each semen parameters and HBA scores over the 5 interval points; Pre-treatment, Treatment 1 and Treatment 2, and Post-treatment 1 and Post-treatment 2. Statistical differences were considered significant, when $P < 0.05$.

3. Results

The mean±SD (range) age of the 12 male subjects who completed this pilot study was 38.6±4.1 (31.2 to 45.2) years. The mean age of their spouses was 37.9±3.7 (32.6 to 46.4) years. The duration of subfertility was 5.1±4.5 (1 to 18) years and 92% ($n = 11$) of the couples were of primary subfertility.

3.1. The effect of acupuncture on semen parameters

The mean±SD semen parameters of these male subjects at Pre-treatment, were: semen volume (3.3±1.9) mL, progressive motility (35.9±8.3) %, vitality (72.3±6.9) %, sperm concentration was (48.8±31.3) million/mL, normal sperm morphology was (1.7±1.2) % and the TMSC (54.4±47.7) million sperm (Table 1). Semen volume, progressive motility, vitality and normal sperm morphology remained relatively constant throughout the study period, from Pre-treatment to the end of Post-treatment 2, (3.3±3.0) mL, (34.8±6.0)

Table 1

Mean semen parameters and sperm-hyaluronan binding assay (HBA) score for the 12 patients on the acupuncture treatment.

| Semen parameters | Pre-treatment | Treatment 1 | Treatment 2 | Post-treatment 1 | Post-treatment 2 | P* |
|---|-------------------------------|-------------------------------|-------------------------------|------------------------------|-----------------------------|------|
| Semen volume (mL) | 3.3 ± 1.9 (1.4 -8.7) | 3.5 ± 2.2 (1.3 -9.6) | 3.4 ± 2.0 (1.3 -9.1) | 3.1 ± 1.6 (0.8 -7.6) | 3.3 ± 3.0 (1.0 -6.8) | NS |
| Progressive motility [(grade a+b), %] | 35.9 ± 8.3 (26 -53) | 37.6 ± 6.7 (28 -48) | 36.5 ± 8.5 (24 -58) | 37.8 ± 7.8 (18 -50) | 34.8 ± 6.0 (24 -45) | NS |
| <i>In situ</i> motility (grade c, %) | 9.3 ± 3.3 (4 -16) | 8.8 ± 3.5 (4 -16) | 9.3 ± 3.7 (4 -19) | 9.1 ± 3.7 (4 -18) | 10.3 ± 4.5 (5 -19) | NS |
| Sperm vitality (%) | 72.3 ± 6.9 (62 -83) | 74 ± 7.9 (55 -84) | 74.42 ± 5.8 (65 -82) | 74.8 ± 76.5 (64 -82) | 70.4 ± 9.1 (55 -82) | NS |
| Sperm concentration (×10 ⁶ sperm/mL) | 48.8 ± 31.3 (19.4 -125.0) | 67.7 ± 30.8 (30.8 -140.0) | 57.3 ± 40.0 (9.4 -147.5) | 62.7 ± 28.4 (22.5 -104.0) | 51.6 ± 25.4 (13.9 -92.0) | NS |
| Normal sperm morphology (%) | 1.7 ± 1.2 (0 -4) | 1.6 ± 0.7 (0 -2) | 1.8 ± 0.8 (1 -3) | 2.0 ± 0.7 (1 -3) | 1.6 ± 0.8 (1 -3) | NS |
| TMSC ×10 ⁶ sperm) | 54.4 ± 47.7 (21.7 - 189.6) | 90.1 ± 68.1 (29.5 - 215.6) | 66.9 ± 68.0 (22.5 - 247.3) | 69.9 ± 42.0 (19.2 -150.2) | 59.7 ± 39.6 (4.4 -132.0) | NS |
| HBA score (%) | 12.0 ± 8.0 (2 -31) | 18.4 ± 11.3 (5 -45) | 16.8 ± 13.9 (2 -45) | 25.8 ± 15.6 (4 -60) | 16.3 ± 12.3 (4 -50) | 0.05 |

HBA, sperm–hyaluronan binding assay; TMSC, total motile sperm count; Data given as mean±SD (range), *P values represent ANOVA with repeated measure, linear trend over time comparisons within-subjects.

%, (70.4±9.1) % and (1.6±0.8) %, respectively.

The means for progressive motility at the 5 treatment intervals for the 12 subjects was relatively constant during the study period. However, acupuncture treatment did have different effects for individual subject. Three subjects (S6, S15 and S18) reacted positively, i.e. the progressive motility increased from Pre-treatment to Post-treatment 2 (Table 2). Negative effect from acupuncture

treatment was observed on patient T4 and T7, the progressive motility decreased from Pre-Treatment to the end of Post-treatment 2, 45% to 32% and 53% to 33%, respectively.

Sperm concentration increased to mean of (67.7±30.8) million/mL at Treatment 1, though was not significantly different compared to the level at Pre-treatment (Table 1). Majority of the subjects, 83% (n =10) had their sperm concentration improved at Treatment 1

Table 2

The effect of acupuncture treatment on progressive motility, concentration and HBA score on individual subjects (n=12).

| Semen characteristics | Subject | Pre-treatment | Treatment 1 | Treatment 2 | Post-treatment 1 | Post-treatment 2 |
|---|---------|---------------|-------------|-------------|------------------|------------------|
| Progressive motility ((grade a+b), %) | T4 | 45 | 30 | 24 | 18 | 32 |
| | T5 | 28 | 29 | 28 | 31 | 32 |
| | S6 | 29 | 36 | 37 | 41 | 37 |
| | T7 | 53 | 28 | 41 | 39 | 33 |
| | S11 | 26 | 43 | 40 | 37 | 29 |
| | T12 | 35 | 35 | 39 | 40 | 34 |
| | S13 | 41 | 44 | 32 | 36 | 41 |
| | S14 | 43 | 44 | 58 | 39 | 36 |
| | S15 | 33 | 43 | 31 | 50 | 45 |
| | S16 | 38 | 36 | 45 | 45 | 31 |
| | S18 | 32 | 35 | 38 | 38 | 43 |
| | S19 | 28 | 48 | 36 | 39 | 24 |
| Sperm concentration (×10 ⁶ sperm/mL) | T4 | 33.1 | 50.0 | 33.9 | 36.8 | 28.9 |
| | T5 | 41.4 | 56.0 | 25.1 | 33.5 | 57.3 |
| | S6 | 20.2 | 30.8 | 32.4 | 64.0 | 59.3 |
| | T7 | 20.0 | 39.0 | 16.3 | 22.5 | 25.3 |
| | S11 | 19.4 | 42.9 | 9.4 | 28.5 | 13.9 |
| | T12 | 32.8 | 57.3 | 51.6 | 45.3 | 76.0 |
| | S13 | 125.0 | 102.5 | 60.0 | 75.5 | 92.0 |
| | S14 | 56.7 | 140.0 | 104.0 | 88.0 | 58.8 |
| | S15 | 49.8 | 83.5 | 90.0 | 71.2 | 50.3 |
| | S16 | 85.7 | 54.8 | 147.5 | 93.0 | 82.5 |
| | S18 | 36.1 | 83.5 | 60.0 | 104.0 | 56.3 |
| | S19 | 65.5 | 71.5 | 57.0 | 90.2 | 18.3 |
| HBA score (%) | T4 | 6 | 6 | 9 | 7 | 35 |
| | T5 | 3 | 5 | 6 | 4 | 7 |
| | S6 | 2 | 5 | 2 | 9 | 11 |
| | T7 | 6 | 17 | 9 | 19 | 6 |
| | S11 | 8 | 15 | 6 | 21 | 4 |
| | T12 | 10 | 15 | 18 | 23 | 10 |
| | S13 | 15 | 20 | 18 | 36 | 18 |
| | S14 | 31 | 22 | 42 | 60 | 50 |
| | S15 | 13 | 18 | 17 | 33 | 20 |
| | S16 | 16 | 24 | 10 | 34 | 24 |
| | S18 | 16 | 29 | 24 | 35 | 17 |
| | S19 | 18 | 45 | 24 | 35 | 11 |

(Table 2). However, the sperm concentration of 8 of these subjects subsequently declined at the end of Treatment 2. The initial sperm concentration of subject S13 declined after 20 sessions of acupuncture treatment from 125.0 to 60.0 million/mL but improved gradually to 92 million/mL 10 weeks after the treatment ended.

The mean TMSC showed similar pattern as the sperm concentration from Pre-treatment to Post-treatment 2 (Table 1). Both the sperm concentration and TMSC reached the highest levels at Treatment 1, declined at the end of the second cycle of acupuncture treatment (Treatment 2), increased slightly at Post-treatment 1, but subsequently decreased at Post-treatment 2.

3.2. The effect of acupuncture on sperm maturity measured by HBA

In this study group, Subject S14 HBA score increased from 31% at Pre-treatment to 22% at 5th week to 42% at 10th week, to 60% at 15th week and 50% at 20th week (Table 2). Another patient, S19, showed improvement from 18% at Pre-treatment to 45% for both 5th and 10th week before declining to 11% at the end of 20th week.

Overall, mean HBA scores was observed to have significant ($P < 0.05$) changes over time. The HBA scores increased from Pre-treatment at 12.0%, to 18.4% ($P = 0.03$) after the 10th acupuncture treatment session and to 16.8% after the 20th session. After treatment, HBA score improved to 25.8% ($P < 0.001$) at Post-treatment 1, but decrease to 16.3% at Post-treatment 2 (Table 1 and Figure 1).

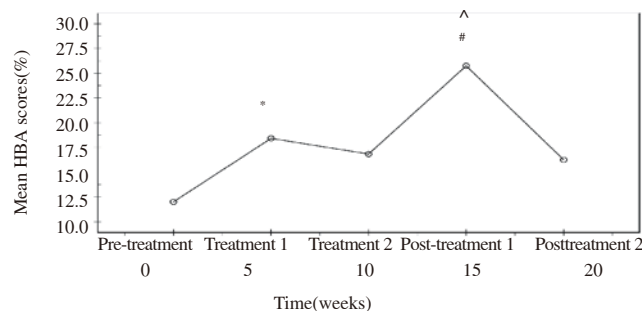


Figure 1. Mean HBA scores at various time points of acupuncture treatment. Mean value of the proportion of mature sperm as measured by sperm-hyaluronan binding assay (HBA) scores. Statistical analysis between different stages of treatment was performed using one-way ANOVA with Bonferroni post hoc analysis. * $P = 0.025$ compared to Pre-treatment, ^ $P < 0.001$ compared to Pre-treatment, # $P = 0.005$ compared to Post-treatment 2.

4. Discussion

In this pilot study, acupuncture has no statistical effect on some of the semen parameters. There were large variations in some semen parameters which differed from Siterman et al, who found improvement in males with very poor sperm concentration, as well as motility and viability [5-7]. This could be because the sperm concentration in our study population was of normal range, >15 million/mL. We also observed that acupuncture treatment might not

be necessary on two subjects (S13 and S16) as it affected their sperm concentration.

In year 2000, Siterman et al showed significant increase of sperm production in some of the males with very poor sperm density, especially those with a history of genital tract inflammation [6]. His group used ‘acupuncture with syndrome diagnosis’ method, by using different combination of main and secondary acupoints for each patient during treatment with different categories of male sterility and syndrome. For ‘deficiency of the kidneys’ and ‘Damp-heat’ syndromes, main points used were *San Yin Jiao*, *Guan Yuan*, *Lie Gue*, *Zho Hai* and *Qi Cong*. For ‘Kidney-yang’ deficiency’ syndrome only, additional main points used were *Tai Xi*, *Shen Shu*, *Heng Hu* and *Zhi Shi*. Five other specific main points for ‘Damp-heat in the genital system’ syndrome only were *Yin Ling Quan*, *Li Gou*, *Qu Chi*, *Shui Dao* and *Zu Li Qi*. Other secondary points were added based on patient’s conditions and syndrome. Similarly in 2009, Siterman et al, used the above treatment regime to treat patients with inflammation of the genital tract associated with scrotal hyperthermia [7]. They were able to observe treatment success in some of their patients.

In our pilot study on the proportion of mature sperm as measured by HBA, there was an increase in the percentage of mature sperm after the acupuncture treatment sessions. Pei J et al showed improvement of sperm quality, specifically in ultra structural integrity of spermatozoa on idiopathic male [9]. However, they reported the percentage of immature sperm by ultra morphologic analysis of spermatozoa, had no statistical significant changes between the control and experimental group, after 5 weeks of treatment. The differences could be due to different methods used for evaluation.

As shown in numerous studies on HBA and hyaluronan-coated dishes (PISI, Biocoat, Fort Washington, PA); bound sperm may have reduced chromosomal abnormalities [10-13]. Our study showed that acupuncture treatment may be able to increase the proportion of mature sperm with reduced chromosomal abnormalities in the semen sample in some patients. We observed that majority of the subjects (83%) with an initial HBA score of <20%, had an increase in the percentage of mature sperm after the first 10 acupuncture treatment sessions. 10 acupuncture sessions might not be beneficial as there were reduction in the HBA scores. However, 5 weeks after the completion of the acupuncture sessions (Post-treatment 1), the HBA scores improved to above 20%. This indicates the possibility of treating men with mild male factor with acupuncture before the assisted reproduction treatment (ART) to reduce the proportion of disomy sperm.

According to TCM, illness is caused by obstructed energy flow at certain points along the meridians. Acupuncture treatment stimulates meridian flow and harmonizes the body’s energy to influence the health of both body and mind. In accordance with the principles of traditional acupuncture and syndrome diagnosis, specific combinations of main points and secondary points are used to treat different patients with different types of conditions

and syndromes. In our pilot study, fixed acupoints were used on all patients, regardless of the type of organ deficiency and syndromes. This could be the reason we observed improvement in motility or density in some patients, while some subjects with inflammation of genital tract were not able to have significant improvement. Perhaps an individualized and customized acupuncture treatment with specific combinations of main and secondary points to treat such patients with specific conditions may yield a better outcome, as the fundamental concept of the Yin and Yang balance in the body is the ultimate therapeutic goal.

In conclusion, this pilot study showed significant linear increase in the mean percentage of mature and functional sperm from Pre-treatment to Post-treatment 1, before decreasing at 10 weeks post-treatment. Thus, acupuncture treatment may have potential positive effect on sperm maturity, quality and fertilizing ability. However, using fixed acupuncture points treatment for all subjects regardless of their fertility factors may not be as effective on the semen parameters as the traditional ‘acupuncture with syndrome diagnosis’ method. More studies are required to fully explore the vast resources of traditional medicines and its efficiency.

Conflict of interest statement

We declare that all authors have no potential conflicts of interest to disclose.

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