Andrologia Volume 32 Page 31 - January 2000 Volume 32 Issue 1

Does acupuncture treatment affect sperm density in males with very low sperm count? A pilot study

S. Siterman, F. Eltes, V. Wolfson, H. Lederman & B. Bartoov

Abstract

Classic therapies are usually ineffective in the treatment of patients with very poor sperm density. The aim of this study was to determine the effect of acupuncture on these males. Semen samples of 20 patients with a history of azoospermia were examined by light microscope (LM) and scanning electron microscope (SEM), with which a microsearch for spermatozoa was carried out. These examinations were performed before and 1 month after acupuncture treatment and revealed that the study group originally contained three severely oligoteratoasthenozoospermic (OTA), two pseudoazoospermic and 15 azoospermic patients. The control group was comprised of 20 untreated males who underwent two semen examinations within a period of 2–4 months and had initial andrological profiles similar to those of the experimental group. No changes in any of the parameters examined were observed in the control group. There was a marked but not significant improvement in the sperm counts of severely OTA males following acupuncture treatment (average=0.7±1.1×10⁶ spermatozoa per ejaculate before treatment vs. $4.3\pm3.2\times10^6$ spermatozoa per ejaculate after treatment). A definite increase in sperm count was detected in the ejaculates of 10 (67%) of the 15 azoospermic patients. Seven of these males exhibited post-treatment spermatozoa that were detected even by LM. The sperm production of these seven males increased significantly, from 0 to an average of $1.5\pm2.4\times10^6$ spermatozoa per ejaculate (Z=-2.8, $P\leq0.01$). Males with genital tract inflammation exhibited the most remarkable improvement in sperm density (on average from $0.3\pm0.6\times10^6$ spermatozoa per ejaculate to $3.3\pm3.2\times10^6$ spermatozoa per ejaculate; Z=-2.4, $P \le 0.02$). Two pregnancies were achieved by the IVF-ICSI procedure. It is concluded that acupuncture may be a useful, nontraumatic treatment for males with very poor sperm density, especially those with a history of genital tract inflammation.

Introduction

Azoospermia is one of the most intractable forms of male infertility. Very few therapeutic regimens have provided effective treatment for azoospermic or severely oligozoospermic patients (Comhaire *et al.*, 1995; Sabanegh & Thomas, 1995; Mantovani *et al.*, 1996; Coppola, 1997; Yong *et al.*, 1997).

In recent years, pregnancies have been achieved by patients suffering from azoospermia by recovery of epididymal or testicular spermatozoa followed by intracytoplasmic sperm injection (Devroey *et al.*, 1995; Silber *et al.*, 1995; Tournaye, 1997; Tournaye *et al.*,

1997, 1998). Although these methods are very effective, they are also invasive, traumatic, technically difficult, require local or general anaesthesia and are genetically problematic (Hirsh *et al.*, 1996; Lisek & Levine, 1997; Tournaye *et al.*, 1997, 1998; de-Wert, 1998).

In parallel to the accepted therapeutic methods, some nonconventional empirical therapies, including Chinese herbal medicine and acupuncture, have also been used in treatment of men with very poor sperm density (Xueying, 1984; Wei, 1988; Ota et al., 1990; Natsuyama et al., 1991).

In a previous study, it was demonstrated that one cycle of acupuncture treatment (twice a week for 5 weeks) may improve sperm parameters of males suffering from subfertility related to low sperm activity (Siterman et al., 1997).

The aim of this prospective, controlled pilot study was to explore the application of the above treatment to males suffering from very low sperm density. Semen samples of males with previously diagnosed azoospermia were observed before and after acupuncture treatment using routine light microscope (LM) and special micro scanning electron microscope (mSEM) examinations.

Materials and Methods

Study groups

Experimental study group.

The experimental study group included 20 patients aged 39±7 years (range 26–48 years) who applied to the Institute of Chinese Medicine for acupuncture treatment due to a history of azoospermia. The azoospermia was diagnosed in different Israeli male fertility laboratories in at least three consecutive LM examinations (with a 3-month interval between examinations). These males had failed to achieve natural pregnancy for an average period of 9±4 years (range 3–13 years). Nineteen of them suffered from primary and only one from secondary infertility. None of the 20 participants in the experimental study group reported smoking or alcohol abuse. None of them had undergone any treatment for at least 1 year prior to acupuncture. All 20 men agreed to participate in the study.

The sperm density of each patient was examined at the Bar-Ilan University Male Fertility Laboratory within 1 month prior to acupuncture treatment. This examination included a routine LM observation and a microsearch for spermatozoa by mSEM. The LM test revealed that three of the 20 participants in this study were severely oligo-terato-asthenozoospermic (OTA). They exhibited between 0.05×10^6 and 1.8×10^6 spermatozoa per ejaculate. Using LM, no sperm cells were observed in the ejaculates of the remaining 17 males. When examined by mSEM, the azoospermia definition was confirmed in 15 of the latter patients. Two remaining patients exhibited 20 and 21 ejaculatory sperm cells, respectively, and were redefined as 'pseudoazoospermic' (Table 1).

The sperm density post-acupuncture was examined 1 month following completion of treatment and included LM and mSEM observations. In addition, semen samples from the men pre- and post-treatment were examined for biochemical, cytological and bacteriological parameters.

Despite the small number of patients, we tried to make this pilot investigation as complete as possible and to evaluate the association between the causes of azoospermia and the response to acupuncture treatment. Accordingly, the 20 participants of the study group were divided into three main aetiological subgroups as follows.

- 1 Six patients, who exhibited high FSH and/or LH levels without any signs of genital tract infection, were defined as suffering from spermatogenic failure (Glezerman & Lunenfeld, 1993). All of these men exhibited hypergonadotrophic hypogonadism. Two underwent testicular biopsy with a diagnosis of 'Sertoli cell only' syndrome.
- 2 Nine patients with normal basal blood FSH and LH levels, who exhibited signs of genital tract infection according to the laboratory criteria, were defined as suffering from inflammation of the genital tract. Seven of these males reported a medical history of prostatitis and another of vesiculitis. Three of these patients reported a history of varicocele. All three severe OTA cases belonged to this category.
- **3** Five other patients, who exhibited high FSH and/or LH levels as well as inflammation of the genital tract, were included in the combined subgroup (spermatogenic failure and genital tract inflammation). Three of these males reported a history of prostatitis, two reported previous varicocele, one had high blood prolactin levels and another suffered from Klinefelter's syndrome. Two of the patients underwent testicular biopsy with a diagnosis of 'Sertoli cell only' syndrome. The testicular biopsy of another patient was defined as 'maturation arrest at the spermatid level' (<u>Table 2</u>).

Untreated control group.

The untreated control group consisted of 20 males who underwent two semen examinations at the Bar-Ilan University male fertility laboratory over 2–4 months. No treatment was performed during this period. In order to exclude the possible association between acupuncture treatment and the original sperm density, the control males were matched with the experimental group according to their andrological profile in the first examination. Thus, the untreated control group also included three males suffering from OTA syndome, two pseudoazoospermic patients and 15 azoospermic males (Table 3). The mean age of these males was 40±9 years (range 29–50 years), which is statistically similar to the age of the experimental group. The semen samples of the untreated control males were twice examined for sperm density as well as for biochemical, cytological and bacteriological parameters.

Treatment

Each patient in the experimental group underwent a total of 10 acupuncture treatments (twice a week for 5 weeks). Sterile disposable stainless steel needles (0.25×25 mm) were inserted in acupuncture point locations. The depth of needle insertion at each point was determined according to the accepted rules of acupuncture treatment (The Cooperative Group of Shandong Medical College and Shandong College of Traditional Chinese Medicine, 1982). Needle reaction (soreness, numbness or distention around the point) was achieved by rotation of the needle. The needles were left in for 25 min and were then removed.

From a traditional 'acupuncture with syndrome diagnosis' perspective, the main causes of male sterility fall under two broad categories: 'deficiency of the kidneys' (usually 'kidney-yang') and 'damp-heat in the genital system' (Maciocia & Kaptchuk, 1998). The former syndrome is usually identical to the spermatogenic failure aetiology and the latter to inflammation of the genital tract.

Acupuncture points appropriate for the 'deficiency of the kidneys' and 'damp-heat' syndromes were regarded as the main points. Points Sp-6 (Sanyinjiao), Ren-4 (GuanYuan), Lu-7 (Liegue), KI-6 (Zhohai) and ST-30 (Qicong) were used for both syndromes. The needles were inserted at these points using the reinforcing method. Four additional specific main points, KI-3 (Taixi), BL-23 (Shenshu), KI-11 (Henggu) and BL-52 (Zhishi), were used for the 'kidney-yang deficiency' syndrome only. These four specific points were also punctured using the above method. Five other specific main points, Sp-9 (Yinlingquan), Liv-5 (Ligou), Li-11 (Quchi), ST-28 (Shuidao) and Gb-41 (Zuliqi), were used only for the 'damp-heat in the genital system' syndrome. The needles were inserted at five points using the reducing method.

The following acupuncture points, which according to the principals of traditional 'acupuncture with syndrome diagnosis' are not associated with the 'kidney-yang deficiency' or 'damp-heat' syndromes, were considered secondary points: LI-4 (Hegu), ST-36 (Zusanli), SP-10 (Xuehai), HT-7 (Shenmen), Bl-20 (Pishu), PC-6 (Neiguan), Ren-1 (Huiyin), Ren-2 (Qugu), Ren-6 (Qihai), Du-4 (Mingmen), Du-20 (Baihui), Gb-20 (Fengchi), Liv-3 (Taichong), KI-7 (Fulu) and Gb-27 (Wushu). Specific combinations of main and secondary points were selected for each patient during treatment according to the principles of traditional 'acupuncture with syndrome diagnosis' (Liangyue *et al.*, 1987; Maciocia & Kaptchuk, 1998). No more than 12 points were punctured during any single session.

Semen analysis

All semen samples were obtained from patients after 17 days of sexual abstinence. The samples were collected by masturbation into sterile condoms suitable for semen analysis.

Evaluation of sperm density

The whole semen sample was first examined for total sperm count using light microscopy according to the WHO standard (WHO, 1992). If no sperm cells were detected, a mSEM test was performed as below.

Upon liquefaction, the fresh ejaculate was centrifuged at 1650 g for 20 min at room temperature (25 °C). The seminal plasma was discarded and the pellet was suspended in 0.5 ml phosphate buffer, 0.1 m, pH 7.4, and recentrifuged at 1650 g for 5 min at room temperature (25 °C). The pellet was resuspended in 0.5 ml of the above phosphate buffer and then fixed with 0.5 ml of 2% (w/v) formaldehyde and 2.5% (v/v) glutaraldehyde in 0.1 m phosphate buffer solution, pH 7.4, for at least 2 h at room temperature (25 °C). An additional centrifugation at 1650 g for 5 min at room temperature (25 °C) was performed post-fixation. The pellet was washed in the above phosphate buffer solution and recentrifuged. The final whole pellet was layered on a round 18-mm glass cover slide pretreated with a 10% poly-L-Lysin solution. Each sample was dehydrated separately with two steps of 50% alcohol, one step of absolute alcohol solution and then one step of 50% freon followed by two steps of 100% freon solutions, air-dried and coated with gold (Cohen, 1974). The entire cover slide was examined by Jeol JSM 840 SEM (Jeol, Welwyn Garden City, UK), first at a magnification of ×3000 and then, when a sperm cell was identified, at a magnification of ×18,000. Mature sperm cells and elongated and early spermatids were counted. The count was stopped when more than 50 mature sperm cells were observed.

In accordance with laboratory quality control examinations, it was found that different semen samples taken from the same ejaculate which exhibited 20 or more sperm cells identified by mSEM had stable sperm counts. Thus, patients originally defined as azoospermic by LM, who exhibited more than 20 sperm cells per ejaculate following a mSEM examination, were confidently redefined as pseudoazoospermic. We could not alter the original andrological definition of the azoospermic patients who exhibited less than 20 spermatozoa per ejaculate, since different semen samples taken from their ejaculates demonstrated high fluctuations in sperm count (between 0 and 19 sperm cells per ejaculate).

Biochemical analysis

Accessory gland function was assessed by measuring seminal Zn²⁺ and Ca²⁺ concentrations as well as citric acid levels, 'prostata markers' and fructose 'seminal vesicle markers'. Zn²⁺ levels were determined by atomic absorption spectrophotometry and the other markers were determined by colorimetric methods (<u>Bartoov et al.</u>, 1993).

Cytological analysis

Cytological tests for white blood cells (WBC), epithelial cells (EPC) and bacteria were performed using the Giemsa staining technique. Numbers of WBC, EPC and bacteria observed were ranked from 0 to 4, based on the impression of the laboratory technician

(<u>Leib et al.</u>, 1994). Patients who exhibited WBC >3 and/or bacteria >3 in cytological tests and/or positive semen culture were considered as suffering from infection of the genital tract. The location of this infection was defined according to the prostate and vesicle markers, the results of prostate and/or seminal vesicle palpation or prostatic secretion.

Statistics

Statistical evaluation was performed using the SPSSx package (Norusis, 1985). Wilcoxon nonparametric tests were used for analysis of sperm density in the experimental group as well as in the untreated control group. Paired *t*-tests were performed for comparison of the biochemical and cytological semen data in each of these groups.

Results

Thirteen out of the 20 members of the experimental group exhibited a considerable improvement in sperm density following acupuncture treatment (Table 1). The three severely OTA males, who underwent one cycle of acupuncture therapy, exhibited a marked but nonsignificant improvement in their sperm count following treatment (average $0.7\pm1.1\times10^6$ spermatozoa per ejaculate before treatment vs. $4.3\pm3.2\times10^6$ spermatozoa per ejaculate after treatment; Table 1, patients 1–3). No substantial improvement in sperm density was observed in the two pseudoazoospermic males (Table 1, patients 4–5). However, 10 (76%) of the 15 azoospermic patients treated by acupuncture exhibited ejaculatory spermatozoa following one cycle of treatment (Table 1, patients 11–20). In three of these patients, more than 20 sperm cells were revealed only by mSEM (Table 1, patients 11–13), while in the seven remaining patients sperm cells could be identified even by LM (Table 1, patients 14–20). The sperm counts of these seven males increased significantly from 0 to an average of $1.5\pm2.4\times10^6$ spermatozoa/ejaculate (Z=-2.8, $P \le 0.01$). None of the untreated controls exhibited any substantial changes in sperm density measured during the second semen examination compared to the first examination (Table 3, patients 1–20).

A very limited effect of acupuncture treatment was observed in the spermatogenic failure subgroup: the andrological profile changed from azoospermic to pseudoazoospermic following one cycle of acupuncture treatment in only two (30%) of the six males included in this category (Tables 1 and 2, patients 11 and 13). In contrast, acupuncture treatment on patients suffering from genital tract inflammation was more effective. Seven (78%) of the nine males included in this subgroup exhibited ejaculatory sperm cells following treatment (Tables 1 and 2; patients 1, 2, 3, 14, 18, 19 and 20), while only three were defined as severely OTA pretreatment (Tables 1 and 2; patients 1, 2 and 3). The average improvement in the sperm production of these males was from $0.3\pm0.6\times10^6$ to $3.3\pm3.2\times10^6$ spermatozoa per ejaculate, which was significant (Z=-2.4, $P\le0.02$). The five patients included in the combined subgroup also responded well to acupuncture treatment: four of them (80%) exhibited a marked increase in their ejaculatory sperm count. Three of them, who were azoospermic before treatment, were defined as severely OTA following acupuncture therapy. They improved their sperm density from 0 to an average of $1.5\pm1.1\times10^6$ spermatozoa per ejaculate (Tables 1 and 2; patients 15–17). The

remaining patient, who was originally azoospermic, exhibited more than 50 sperm cells which were detected only by mSEM following treatment (Tables 1 and 2; patient 12).

No changes in the basal hormonal blood levels were observed in any of the participants of the experimental study group.

No significant changes were observed post-acupuncture therapy in the biochemical markers of the treated males. It should be mentioned that the average values of these parameters were normal prior to treatment as well as after acupuncture therapy. Regarding cytological semen parameters, the treated patients exhibited initially high levels of white blood cells and bacteria. None of these parameters was affected by the acupuncture treatment (Table 5). Only two of the 14 members of the experimental group with a positive semen culture exhibited negative semen culture post-treatment. No differences were observed in the untreated control group regarding the biochemical or cytological parameters of the first and second examinations.

Two men in the inflammation subgroup underwent two additional acupuncture procedures, and exhibited a continuous improvement in their sperm density (<u>Table 4</u>; patients 14 and 19).

Only two of the 20 treated patients underwent ICSI treatment following the acupuncture procedure (patients 17 and 19). Pregnancies were achieved in both cases. The female partner of patient 19 underwent a spontaneous abortion.

Discussion

Acupuncture is an empirical therapy, which has been demonstrated to improve sperm parameters of males suffering from impaired sperm quality (Shealy et al., 1990; Gerhard et al., 1992; Siterman et al., 1997).

The present pilot study shows that this treatment may also have a positive effect on sperm production in males with very poor sperm density. Indeed, a distinct increase in sperm count following acupuncture therapy was detected in the ejaculates of 13 (65%) out of 20 treated males, while no changes in this parameter were observed in any of the untreated controls. Furthermore, 41% of the initially azoospermic patients exhibited spermatozoa following acupuncture treatment, which were detected even by LM. These results are in complete agreement with those of Xueying (1984), who reported the appearance of sperm cells following one cycle of acupuncture treatment in 125 out of 160 azoospermic men. However, the above investigation had very limited data and was uncontrolled.

Regarding control investigations, it would be of great interest to treat azoospermic patients by choosing acupuncture points which are not related to male fertility and only then, following 1 month of so-called 'placebo treatment', to perform the 'real treatment', choosing specific points. On the other hand, since each acupuncture point has a specific effect on the human body, no acupuncture point can be considered a 'placebo' point (Liangyue *et al.*, 1987; Maciocia & Kaptchuk, 1998). Thus, performance of a highly

controlled investigation of this kind presents an ethical problem in human populations. Furthermore, it is not possible to perform double-blind studies.

From two cases reported in this study it seems that continuation of the acupuncture treatment for at least three cycles may lead to an additional improvement in sperm density. This effect should be further investigated in a larger number of patients.

Since the mechanism of acupuncture treatments has not been revealed, we tried to define the appropriate responders to this treatment using aetiological criteria for poor sperm density. We are aware of the fact that a very small number of patients participated in this pilot investigation and therefore definite conclusions cannot be reached. However, according to the very preliminary results, it seems that azoospermic and/or severely oligozoospermic males suffering from genital tract inflammation exhibited the greatest improvement in sperm density following acupuncture treatment. However, none of these men exhibited any changes in biochemical or cytological parameters associated with the above aetiology. Semen culture was also found to be an almost unaffected parameter: only two of 14 patients with positive semen culture improved following treatment. These results confirm the findings of our previous study, which demonstrated that no biochemical, cytological or bacteriological semen parameters were improved following acupuncture treatment in males with low sperm quality (Siterman et al., 1997). It should be emphasized that studies claiming that acupuncture has an excellent clinical efficacy in treating chronic prostatitis reported improvement in the clinical symptoms of the treated males but did not mention any laboratory results (Ge et al., 1988; Benre et al., 1990). The claim of Tang et al. (1996) that acupuncture could reduce a local inflammatory reaction by enhancing immune responses seems to be an acceptable explanation for this phenomenon.

In order to confirm that males suffering from genital tract inflammation are the most appropriate candidates for acupuncture treatment, a further study with large groups of patients with clearly defined aetiological factors should be performed. A placebo group using the five acupuncture points specific for the inflammation patients should be considered in the protocol of such a study.

Acupuncture treatment is simple, noninvasive and inexpensive, and does not require any previous preparation, so seems to be clinically preferable to sperm aspiration and extraction from the testis or epididymis. The fertility potential of spermatozoa achieved by acupuncture treatment in azoospermic males will be investigated in the near future.

Tables

Table 1 Sperm count before and after one cycle of acupuncture treatment as obtained by LM and mSEM (n=20)

Patient	Andrological definition pretreatment	Sperm count	Andrological definition				
		$ \begin{array}{l} {\rm LM} \\ (\times 10^6 \; {\rm spermatozoa/ejaculate}) \end{array} $		mSEM (sperm cells/ejaculate)		post-treatmen	
		Pretreatment	Post-treatment	Pretreatment	Post-treatment		
1	OTA	0.05	7.5	nd	nd	OTA	
2	OTA	0.06	1	nd	nd	OTA	
3	OTA	1.8	4.5	nd	nd	OTA	
4	PA	0	0	20	20	PA	
5	PA	0	0	21	1	A	
6	A	0	0	0	0	A	
7	A	0	0	0	l + lsp	A	
8	A	0	0	14sp	2 + 2sp	A	
9	A	0	0	0	4 + 6sp	A	
10	A	0	0	1 + 1	5 + 32sp	A	
11	A	0	0	3 + 6sp	>50	PA	
12	A	0	0	0	>50	PA	
13	A	0	0	0	> 50	PA	
14	A	0	0.01	0	nd	OTA	
15	A	0	0.03	0	nd	OTA	
16	A	0	0.04	3	nd	OTA	
17	A	0	0.3	0	nd	OTA	
18	A	0	0.8	0	nd	OTA	
19	A	0	1.3	2	nd	OTA	
20	A	0	7.5	0	nd	OTA	

Table 2 Clinical and andrological definition of the study group before and after acupuncture treatment

Patient			Clinical definition	Andrological definition*		
Seq. No.	Age	Pregnancy expectation (years)	Category	Medical history†	Preteatment	Post-treatment
5	31	3	Spermatogenic failure	HFSH, HLH, Hypopl., Var.	PA	A
7	36	5		HFSH, HLH, Hypopl.	A	A
9	48	13		HFSH, HLH, Sert. cell only, Hypopl.	A	A
10	27	3		HFSH, HLH, Hypopl.	A	A
11	39	10		HFSH, HLH, Hypopl.	A	PA
13	29	5		HFSH, HLH, Sert. cell only, Crypto., Hypopl.	A	PA
6	39	15	Inflammation of the genital tract	Nhor., Var., Prost.	A	A
8	39	13		Nhor., Prost.	T	T
3	35	11		Nhor., Prost., Var.	OTA	OTA
2	33	10		Nhor., Crypto., Vesicul., Prost.	OTA	OTA
14:	39	13		Nhor.	A	OTA
18	41	8		Nhor., Prost., Var.	A	OTA
1§	48	5		Nhor.	OTA	OTA
20	25	2		Nhor., Prost.	A	OTA
19‡¶	37	5		Nhor., Prost., Var.	A	OTA
4	38	12	Combined	HFSH, HLH, Var., Prost.	PA	PA
12	26	3		HFSH, NLH, Sert. cell only, Var.	A	PA
15	35	12		HFSH, NLH, Sert. cell only.	A	OTA
16	30	5		HFSH, NLH, Late matur. Arr., Prost., Hprol.	A	OTA
17¶	32	8		HFSH, Prost., Klinefelter	A	OTA

*OTA, oligoteratoasthenozoospermia; PA, pseudoazoospermic; A, azoospermia.
†HFSH, high FSH; HLH, high LH; Hypopl., testicular hypoplasia; Var., varicocele; Sert. cell only, Sertoli cell only syndrome; Crypto., cryptorchidism; NHor., normal hormonal level blood; Prost., chronic prostatitis; Vesic., chronic vesiculitis; Hprol., high prolactine level.
‡Continued treatment for two additional cycles (See Table 3).
\$Secondary infertility.

¶Pregnancy achieved following IVF-ICSI.

Table 3 Sperm count in the first and second semen examinations in the untreated control group as obtained by LM and mSEM (n=20)

Patient	Andrological definition Exam. 1	Sperm count	Andrologica definition				
		LM (×10 ⁶ spermatozoa/ejaculate)		mSEM (sperm cells/ejaculate)		Exam. 2	
		Exam. 1	Exam. 2	Exam. 1	Exam. 2		
1	OTA	8.1	7.2	nd	nd	OTA	
2	OTA	0.62	80.0	nd	nd	OTA	
3	OTA	0.62	1.55	nd	nd	OTA	
4	PA	0	0	20	10	A	
5	PA	0	0	40	30	PA	
6	A	0	0	0	0	A	
7	A	0	0	0	0	A	
8	A	0	0	0	0	A	
9	A	0	0	0	0	A	
10	A	0	0	3	5	A	
11	A	0	0	3 + 2sp	5	A	
12	A	0	0	0	8	A	
13	A	0	0	4	2	A	
14	A	0	0	7	6	A	
15	A	0	0	10	8	A	
16	A	0	0	10	16	A	
17	A	0	0	10	18	A	
18	A	0	0	13	16	A	
19	A	0	0	15	8	A	
20	A	0	0	16	12	A	

Table 4 Sperm count per ejaculate in two patients before and after three acupuncture treatment cycles

Patient	LM				mSEM			
	Pre	Cycle I	Cycle II	Cycle III	Pre	Cycle I	Cycle II	Cycle III
14	0	0.01×10^{6}	0.03 × 10 ⁶	0.1×10^{6}	0	nd	nd	nd
19	0	1.3×10^{6}	3.8×10^{6}	8.8×10^{6}	2	nd	nd	nd

Table 5 Mean values of biochemical and cytological semen parameters before and after acupuncture treatment (*n*=20)

Semen parameters	Lab. standard values	Pretreatment (M±SD)	Post-treatment $(M \pm SD)$
Volume (ml)	1.5-6.0	4.1 ± 2.4	4.3 ± 2.6
Ca ²⁺ (mg percentage)	7.4-26.0	20.0 ± 9.7	19.5 ± 10.9
Fructose (mg percentage)	120-600	215.1 ± 118.0	211.2 ± 116.6
$Zn^{2+} (\mu g m l^{-1})$	80-230	113.1 ± 53.2	122.4 ± 61.9
Citric acid (mg percentage)	349-671	518.4 ± 365.4	528.0 ± 437.7
White blood cells (ranked 1-4)	≤2	2.3 ± 1.8	2.4 ± 1.5
Epithelial cells (ranked 1-4)	≤2	1.8 ± 1.6	1.7 ± 2.0
Bacteria (ranked 1-4)	≤2	2.3 ± 1.3	2.1 ± 1.3

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Affiliations

¹Institute of Chinese Medicine, Tel Aviv, Israel, ²Male Fertility Laboratory, Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

Correspondence

Correspondence to: Prof. B. Bartoov Male Fertility Laboratory, Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel. Tel.: 972–3-5318219; Fax: 972–3-5343679.