

## Original Article

# Success of acupuncture treatment in patients with initially low sperm output is associated with a decrease in scrotal skin temperature

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## Abstract

Poor spermatogenesis in patients with inflammation of the genital tract is associated with scrotal hyperthermia. These patients can benefit from acupuncture treatment. We conducted a study to verify whether the influence of acupuncture treatment on sperm output in patients with low sperm density is associated with a decrease in scrotal temperature. The experimental group included 39 men who were referred for acupuncture owing to low sperm output. The control group, which comprised 18 normal fertile men, was used to define a threshold (30.5°C) above which scrotal skin temperature was considered to be high. Accordingly, 34 of the 39 participants in the experimental group initially had high scrotal skin temperature; the other five had normal values. Scrotal skin temperature and sperm concentration were measured before and after acupuncture treatment. The five patients with initially normal scrotal temperatures were not affected by the acupuncture treatment. Following treatment, 17 of the 34 patients with hyperthermia, all of whom had genital tract inflammation, had normal scrotal skin temperature; in 15 of these 17 patients, sperm count was increased. In the remaining 17 men with scrotal hyperthermia, neither scrotal skin temperature nor sperm concentration was affected by the treatment. About 90% of the latter patients suffered from high gonadotropins or mixed etiological factors. Low sperm count in patients with inflammation of the genital tract seems to be associated with scrotal hyperthermia, and, consequently, acupuncture treatment is recommended for these men.

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## 1 Introduction

The concept that an elevation of scrotal skin temperature results in impairment of spermatogenesis is widely accepted [1–4]. Several authors believe that scrotal hyperthermia is a modern lifestyle condition associated with tight-fitting, thermally insulating clothing [5], sedentary work (especially driving a vehicle for prolonged periods) [6], obesity [7], exposure

to electromagnetic waves [7] and use of a laptop computer [8]. Studies performed in bulls showed that dietary energy [9] and gonadotropin-releasing hormone (GnRH) treatment [10] may also increase scrotal skin temperature.

Studies of infertile men with low sperm output have consistently shown that they have higher scrotal temperatures compared with fertile controls [11–14]. Over the years, the inverse correlation between sperm output and hyperthermia has prompted a number of therapeutic options for the reduction of scrotal temperature, such as an evaporative cooling device [14], scrotal cooling by applying ice packs [15] and nocturnal scrotal cooling by periscrotal air circulation [13, 16].

In our earlier controlled study, we showed that acupuncture treatment is an additional therapeutic method that improves sperm output in men suffering from azoospermia or severe oligozoospermia [17]. The aim of this study was to verify whether the influence of acupuncture treatment on sperm output is associated with a decrease in scrotal temperature.

## 2 Materials and methods

### 2.1 Experimental group

The experimental group comprised 39 primarily infertile men who were referred for acupuncture treatment and who agreed to participate in this study. Their average age was  $36.5 \pm 3.4$  years. Twenty-six of the infertile men were defined as azoospermic (total sperm count = 0), nine others had severe oligozoospermia ( $0 < \text{sperm concentration} < 5 \times 10^6$  spermatozoa per mL) and the remaining four were diagnosed as oligozoospermic ( $5 \times 10^6 \leq \text{sperm concentration} < 20 \times 10^6$  spermatozoa per mL, Table 1). A low sperm concentration in each patient was confirmed by at least three consecutive routine semen examinations. The last sperm examination was performed at the Bar Ilan Male Fertility Laboratory (Ramat Gan, Israel) not more than one month prior to the commencement of acupuncture treatment.

Thirteen participants in the experimental group had more than  $10^6$  white blood cells (WBC) in their ejaculates and positive semen culture and were therefore defined as suffering from an inflammation of the genital tract. Thirteen other patients had high levels of follicle stimulating hormone (FSH) compared with local laboratory standards. A mixed etiology of inflammation of the genital tract and high FSH levels was obtained in

four men. One patient suffered from isolated palpable grade II varicocele, and eight other participants in the experimental group had a mixed etiology of palpable grade II or III left varicocele and inflammation of the genital tract. One patient had undergone radiation therapy 10 years earlier (Table 1).

None of the 39 participants in the experimental study group had any surgical pathology or had undergone any treatment, including antibiotic treatment, for inflammation of the genital tract. All participants said they had never smoked or taken drugs. The mean pregnancy expectation time in the experimental group was  $4.3 \pm 2.6$  years.

### 2.2 Experimental subgroups

Following analysis of the distribution of scrotal skin temperatures in the control group,  $30.5^\circ\text{C}$  was defined as the cutoff point between normal and high values for this parameter. Subsequently, the patients included in the experimental study group were subdivided into three subgroups—A, B and C—according to their mean scrotal skin temperatures before and after acupuncture treatment (see the Results section).

### 2.3 Control group

Eighteen normal control volunteers, aged  $36.6 \pm 5.6$  years, were selected according to the following criteria: spontaneous pregnancy achieved within not more than 1 year of pregnancy expectation, with the last pregnancy not more than 1 year prior to examination; no diagnosed varicocele; and no pathology in the urogenital system. All volunteers said they had never smoked or taken drugs.

### 2.4 Acupuncture treatment

Each patient in the experimental group underwent a course of 8–10 acupunctural treatments (two treatments a week). The treatment was finished when, by the criteria of Chinese medicine, an energetic balance was achieved, and, in parallel, the patient experienced a permanent feeling of very heavy testicles.

Sterile disposable stainless steel needles ( $0.25 \text{ mm} \times 25 \text{ mm}$ ) were inserted in acupuncture point locations. The depth of needle insertion at each point was determined according to the accepted rules of the acupuncture treatment (the Cooperative Group of Shandong Medical College and Shandong College of Traditional Chinese Medicine, 1982). Rotation of the needle caused soreness, numbness or distention around the point. The

Table 1. Association between sperm concentration and scrotal skin temperature before and after acupuncture treatment.

Subgroup	Patient	Etiological profile	Before acupuncture treatment				After acupuncture treatment			
			Temp. category	Scrotal skin temp.	Androl. profile	Sperm concentration per mL	Temp. category	Scrotal skin temp.	Sperm concentration per mL	Treatment success
A (n = 5)	1	hFSH	N	30.25	AZO	0	N	30.23	0	NO
	2	hFSH	N	30.47	AZO	0	N	30.17	0	NO
	3	hFSH	N	30.00	AZO	0	N	30.05	0	NO
	4	hFSH	N	30.33	AZO	0	N	30.35	0	NO
	5	hFSH	N	30.44	AZO	0	N	30.42	0	NO
B (n = 17)	6	IN+hFSH	H	30.58	AZO	0	H	30.69	0	NO
	7	hFSH	H	31.30	AZO	0	H	30.76	0	NO
	8	hFSH	H	31.15	AZO	0	H	31.02	0	NO
	9	IN+hFSH	H	31.02	AZO	0	H	31.35	0	NO
	10	hFSH	H	31.62	AZO	0	H	31.33	0	NO
	11	hFSH	H	31.51	AZO	0	H	30.88	0	NO
	12	IN+V	H	31.01	AZO	0	H	30.95	0	NO
	13	IN	H	30.74	AZO	0	H	31.07	0	NO
	14	IN+V	H	31.28	AZO	0	H	31.54	0	NO
	15	IN+V	H	31.22	AZO	0	H	31.09	0	NO
	16	IN	H	30.63	AZO	0	H	30.75	40*	NO
	17	RA	H	31.10	AZO	0	H	31.24	50*	NO
	18	hFSH	H	31.19	AZO	0	H	31.05	80*	NO
	19	IN+V	H	30.63	SO	$2.5 \times 10^6$	H	30.66	$2.3 \times 10^6$	NO
	20	V	H	30.87	O	$5 \times 10^6$	H	31.30	$4.3 \times 10^6$	NO
	21	IN+V	H	31.76	SO	$0.7 \times 10^6$	H	31.65	$4 \times 10^6$	NO
	22	IN+V	H	31.54	O	$7 \times 10^6$	H	31.06	$0.8 \times 10^6$	NO
C (n = 17)	23	IN	H	30.59	AZO	0	N	30.08	50 <sup>MSOME</sup>	NO
	24	IN	H	30.75	O	$8 \times 10^6$	N	29.22	$8.7 \times 10^6$	NO
	25	IN	H	30.62	AZO	0	N	29.67	180*	Yes
	26	IN+V	H	31.12	AZO	0	N	29.39	180*	Yes
	27	IN	H	30.73	AZO	0	N	29.50	350*	Yes
	28	IN	H	30.59	AZO	0	N	30.39	500*	Yes
	29	IN+hFSH	H	31.00	AZO	0	N	30.43	$3 \times 10^3$	Yes
	30	IN+hFSH	H	30.77	AZO	0	N	29.99	$4 \times 10^4$	Yes
	31	IN	H	30.86	AZO	50	N	30.45	$3 \times 10^6$	Yes
	32	IN	H	30.80	SO	$1.2 \times 10^6$	N	29.72	$48 \times 10^6$	Yes
	33	IN	H	31.44	SO	$1.3 \times 10^6$	N	30.41	$28 \times 10^6$	Yes
	34	IN	H	30.79	SO	$1.4 \times 10^6$	N	30.12	$9 \times 10^6$	Yes
	35	IN	H	30.80	SO	$2 \times 10^6$	N	29.89	$5.2 \times 10^6$	Yes
	36	IN	H	30.89	SO	$2.1 \times 10^6$	N	29.81	$10.0 \times 10^6$	Yes
	37	IN	H	30.63	SO	$3 \times 10^6$	N	29.24	$15 \times 10^6$	Yes
	38	IN+V	H	30.78	SO	$4.5 \times 10^6$	N	29.86	$12.2 \times 10^6$	Yes
	39	IN	H	30.75	O	$9.2 \times 10^6$	N	30.24	$45.0 \times 10^6$	Yes

Abbreviations: AZO, azoospermia; H, high scrotal temperature; hFSH, high follicle stimulating hormone level; IN, inflammation of the genital tract; MSOME, Motile Sperm Organelle Morphology Examination; N, normal scrotal temperature; O, oligozoospermia; R, radiation; SO, severe oligozoospermia; V, varicocele. \*Number of sperm cells per ejaculate observed by MSOME.

needles were left in for 25 min and then removed.

In accordance with the principles of traditional acupuncture and syndrome diagnosis [18], acupuncture points appropriate for the deficiency of the kidneys (hormonal imbalance) and damp-heat syndromes (inflammation of the genital tract) 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. Four additional main points—KI-3 (Taixi), BL-23 (Shenshu), KI-11 (Henggu) and BL-52 (Zhishi)—were used only for the kidney-yang deficiency syndrome (spermatogenic failure). At all these points, the needles were inserted using the reinforcing method.

Five other main points—Sp-9 (Yinlingquan), Liv-5 (Ligou), Li-11 (Quchi), ST-28 (Shuidao) and Gb-41 (Zuliqi)—were used only for damp-heat in the genital system (inflammation of the genital tract). The needles were inserted at five points using the reducing method. The following acupuncture points, which, according to the principles of traditional acupuncture, 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 [18]. No more than 12 points were punctured during any single session.

### 2.5 Measurement of femoral and scrotal skin temperatures

Temperature measurement was performed with the Cont-Flex m<sup>®</sup> system (IPS, Milan, Italy), using the plate in accordance with the manufacturer's instructions. The temperature of the femur and the temperatures of the left and right hemiscrota in the experimental group were evaluated prior to the first acupuncture treatment for the initial values and prior to the last acupuncture treatment for post-treatment values. These evaluations were performed with the patient lying on his back, undressed, with legs spread apart and bent at the knees, after remaining for 10 min at a room temperature of 22–24°C. In accordance with the manufacturer's instructions for identifying

the appropriate thermographic plate to be used for obtaining simultaneous images, a plate was placed on the scrotum and the upper interior part of the femur. If it displayed the intermediate colors (green, light blue or violet), the plate had the correct thermal sensitivity for thermographic measurement. We found the thermographic plate RW 29 ST (IPS, Milan, Italy), with color changes at 28.3°C, 29.0°C, 29.5°C, 30.1°C, 30.6°C, 31.3°C, 32.3°C and 33.3°C, to be the one most suitable for our purposes. Indeed, the values obtained with this plate conform to the expected scrotal temperatures of healthy adult men after physiological cooling (28–32°C), according to the manufacturer's instructions. We adapted a digital Nikon E 950 Coolpix 950 photo camera (Nikon Corporation Imaging Products Division Shinagawa-Ku, Tokyo, Japan) instead of the traditionally used Polaroid Image 2 system. The pictures taken by this camera were analyzed using a special computer program developed in our laboratory. The software, which was designed to allow a quantitative analysis of the patient's condition using thermographic data, provides two major components: data extraction and data storage/analysis. The extraction consists of the processing of a digital thermal image, extraction of a temperature average and breakdown of a specified region. This computer program enabled us to calculate the femoral temperature and the temperatures of each hemiscrota.

No statistical difference was obtained between the skin temperatures of the left and right hemiscrota in any of the studied individuals. The average of these two values was thus considered to be the mean scrotal skin temperature.

To verify the degree of reproducibility in temperature measurement, six control men were examined five times each for their scrotal and femur temperatures, with a time interval of 10 min. The mean values of femur and scrotal temperatures in the 39 measurements were  $30.01 \pm 0.69^\circ\text{C}$  (SE of mean =  $0.08^\circ\text{C}$ ) and  $30.74 \pm 0.29^\circ\text{C}$  (SE of mean =  $0.04^\circ\text{C}$ ), respectively. The alpha model of reliability analysis showed that the five measures of these two parameters were reproducible ( $\alpha = 0.92$ , and  $\alpha = 0.94$ , respectively).

Femur and scrotal skin temperatures in the control group were first evaluated in all 18 control men. Only four agreed to repeat this procedure after a month; however, as no significant difference was obtained between the former and the latter measures with regard to each of the initial parameters, one measurement for

all 18 controls was considered sufficient.

### 2.6 Sperm concentration

As the cycle of seminiferous epithelium in humans is 16 days, the semen samples from patients with azoospermia were obtained after 17 days of sexual abstinence. Semen samples from patients with severe oligozoospermia and oligozoospermia were obtained after 4 days of sexual abstinence, according to the World Health Organization (WHO) guidelines [19]. The samples were collected through masturbation into sterile condoms without spermicide.

The whole semen sample was first examined for total sperm count using routine light microscopy (LM) [19]. When no sperm cells were found by this method (azoospermia), a high-power ( $\times 6\ 300$ ) examination, using an inverted light microscope equipped with Nomarski optics, enhanced by digital imaging, was performed [20]. For this purpose, each milliliter of the native semen was gently placed on a two-layer Sil-Select density gradient, which consists of 1 mL upper (low density) and 0.1 mL lower (high density) layers of silane-coated colloidal silica particles suspended in HEPES-buffered Earle's balanced salt solution (EBSS; Ghent, Belgium). Centrifugation at  $375 \times g$  for 15 min at  $25^\circ\text{C}$  was performed. The sperm cell pellet was suspended by adding 1 mL of SPERM medium (Medi-Cult, Jyllinge, Denmark) and then re-centrifuged at  $375 \times g$  for 10 min at  $25^\circ\text{C}$ . The final sperm pellet obtained was gently re-suspended in about 20–50  $\mu\text{L}$  of SPERM medium for observation using a high-power microscope.

The search for sperm cells was conducted in a glass-bottomed Petri dish [21]. In all, 2–4 mL of the final sperm suspension was added to each of 4–8 microdroplets of 10  $\mu\text{mL}$ . The SPERM medium was supplemented with 20% human albumin (D-35002; Behring GmbH, Marburg, Germany).

Acupuncture treatment was considered successful if at least one of the following occurred: (a) 100 sperm cells could be obtained by Motile Sperm Organelle Morphology Examination (MSOME) in the ejaculates of initially azoospermic patients; (b) a few spermatozoa could be obtained by LM in the ejaculates of patients in whom sperm cells could initially be observed only by MSOME; (c) severe oligozoospermia was defined in patients who initially had only a few spermatozoa obtained by LM; (d) oligozoospermia was defined in patients who initially suffered from severe oligozoosper-

mia; and (e) normal sperm concentration was obtained in patients previously defined as oligozoospermic.

### 2.7 Statistical analysis

All statistical analyses were performed using SPSS for Windows version 10.0 (SPSS Inc., Chicago, IL, USA). Data were presented as mean  $\pm$  SD. Comparison between the control and infertile groups was performed by one-way analysis of variance (ANOVA) tests. Comparison between pre- and post-treatment conditions in the infertile group was performed using paired *t*-tests. Because of the abnormal distribution of the scrotal skin temperature in subgroups A and C (see Results section), comparisons within these subgroups were performed by Wilcoxon signed ranks tests using non-parametric statistics. Comparisons between the three subgroups were performed using Mann-Whitney non-parametric statistics.

## 3 Results

The average femur temperatures in the fertile control group and the experimental group before and after acupuncture treatment were statistically similar ( $31.06 \pm 0.41^\circ\text{C}$ ;  $31.07 \pm 0.44^\circ\text{C}$ ; and  $30.96 \pm 0.57^\circ\text{C}$ , respectively).

The distribution of the scrotal skin temperature in the control group is shown in Figure 1A. The mean value of this parameter in the fertile men was  $29.78 \pm 0.36^\circ\text{C}$ , with a median of  $29.80^\circ\text{C}$ . As this distribution was normal, the threshold between the normal and elevated scrotal skin temperatures, calculated for the population of this study, was defined to be  $30.50^\circ\text{C}$  (mean  $\pm$  SD). Thus, scrotal skin temperature  $> 30.50^\circ\text{C}$  was considered high.

Prior to the acupuncture treatment, the experimental group had a significantly higher average scrotal skin temperature compared with the control group ( $30.87 \pm 0.37^\circ\text{C}$  vs.  $29.78 \pm 0.36^\circ\text{C}$ ,  $F = 125.0$ ,  $P < 0.01$ , Table 2, Figures 1A and 1B). After treatment, the average scrotal skin temperature in the experimental group was significantly reduced compared with the initial measurement ( $30.45 \pm 0.63^\circ\text{C}$  vs.  $30.88 \pm 0.39^\circ\text{C}$ ,  $t = 4.8$ ,  $P < 0.01$ , Table 2, Figure 1C). Nonetheless, it was still significantly higher than the average scrotal skin temperature in the normal control group ( $30.45 \pm 0.63^\circ\text{C}$  vs.  $29.84 \pm 0.33^\circ\text{C}$ ,  $F = 9.0$ ,  $P < 0.01$ , Table 2, Figure 1C).

Using the calculated threshold, 34 of 39 (87.1%) members in the experimental group were defined as

Table 2. Scrotal skin and femoral temperatures in the control-fertile group, the experimental group and the three subgroups before and after acupuncture treatment.

	Scrotal skin temperature (°C)	Femur temperature (°C)
Control ( <i>n</i> = 18)	29.78 ± 0.36	31.06 ± 0.41
Experimental study groups ( <i>n</i> = 39)		
Pre	30.87 ± 0.37 <sup>a</sup>	31.07 ± 0.44
Post	30.45 ± 0.63 <sup>a,b</sup>	30.96 ± 0.57
Subgroups of the experimental study group		
A ( <i>n</i> = 5)		
Pre	30.32 ± 0.20 <sup>c</sup>	31.13 ± 0.41
Post	30.04 ± 0.28 <sup>c</sup>	30.90 ± 0.73
B ( <i>n</i> = 17)		
Pre	31.16 ± 0.30 <sup>d</sup>	31.00 ± 0.48
Post	31.10 ± 0.27	31.02 ± 0.48
C ( <i>n</i> = 17)		
Pre	30.81 ± 0.23	31.07 ± 0.16
Post	29.90 ± 0.40 <sup>b</sup>	31.00 ± 0.63

<sup>a</sup>Significantly higher compared with the scrotal skin temperature in the control group (*P* < 0.01);

<sup>b</sup>Significantly lower compared with the pre-treatment condition (*P* < 0.01);

<sup>c</sup>Significantly lower compared with the pre-treatment condition in subgroup C (*P* < 0.01);

<sup>d</sup>Significantly higher compared with the pre-treatment condition in subgroup C (*P* < 0.01);

<sup>e</sup>Significantly higher compared with the post-treatment condition in subgroup C (*P* < 0.01).

having a high scrotal temperature prior to treatment, and the other five had normal values of this parameter. When the same threshold was applied to the post-treatment scrotal skin temperature values in the experimental group, the patients could be divided into three subgroups: subgroup A—five patients with normal scrotal skin temperature before and after acupuncture treatment (patients 1–5, Table 1, Figure 2A); subgroup B—17 men with elevated scrotal skin temperature before and after treatment (patients 6–22, Table 1, Figure 2B); and subgroup C—17 patients who had scrotal hyperthermia before treatment and normal scrotal skin temperature after treatment (patients 23–39, Table 1, Figure 2C).

Within subgroups A and B, the average values of the scrotal skin temperature before and after treatment were statistically similar (Table 2, Figures 2A and 2B), whereas within subgroup C, the pre-treatment scrotal skin temperature was significantly higher than the post-

treatment value (30.81 ± 0.23°C vs. 29.90 ± 0.41°C, *Z* = 3.6; *P* < 0.01, Table 2, Figure 2C). Comparison between subgroup C and the two other subgroups with regard to their pre-treatment average scrotal skin temperatures showed that the initial value of this parameter in subgroup C was significantly higher than that in subgroup A (30.81 ± 0.23°C vs. 30.32 ± 0.20°C: *Z* = 2.9; *P* < 0.01) and significantly lower than that in subgroup B (30.81 ± 0.25°C vs. 31.16 ± 0.30°C: respectively, *Z* = -3.3; *P* < 0.01, Table 2). Comparison of the post-treatment average scrotal skin temperatures showed that the value of this parameter in subgroup C was statistically similar to that in subgroup A and significantly lower than that in subgroup B (29.90 ± 0.41°C vs. 31.10 ± 0.27°C, *Z* = 3.3, *P* < 0.01, Table 2).

With respect to the etiological profile of the three subgroups, it was found that all five patients in subgroup A had high FSH levels only (Table 1). In subgroup B, nine patients suffered from isolated etiological factors, including five patients with high FSH levels (patients 7, 8, 10, 11, and 18); two patients with inflammation of the genital tract (patients 13 and 16); one man with varicocele (patient 20); and one man who had been treated with radiation 10 years previously (patient 17, Table 1). The other eight patients in subgroup B had mixed etiological factors, including two patients with high FSH levels and inflammation of the genital tract (patients 6 and 9) and six patients with inflammation of the genital tract and varicocele. In subgroup C, all 17 patients had inflammation of the genital tract, including two men who additionally had high FSH levels (patients 29 and 30) and two men with additionally diagnosed varicocele (patients 26 and 38).

With respect to the success of the acupuncture treatment in increasing sperm output, none of the patients in the A and B subgroups had improvement in their sperm concentration following acupuncture treatment (Table 1). However, 15 (88.2%) of the 17 men in subgroup C had an increased sperm concentration following acupuncture treatment. Of the seven initially azoospermic men, four had ≥ 100 sperm cells in their ejaculates (patients 25–28) and three others became severely oligozoospermic (patients 28–31, Table 1). Of the seven initially severe oligozoospermic patients, two had normal sperm concentration (patients 32–33) and five became oligozoospermic (patients 34–38, Table 1) following acupuncture treatment. The last oligozoospermic man also had normal sperm concentration (patient 39, Table 1).

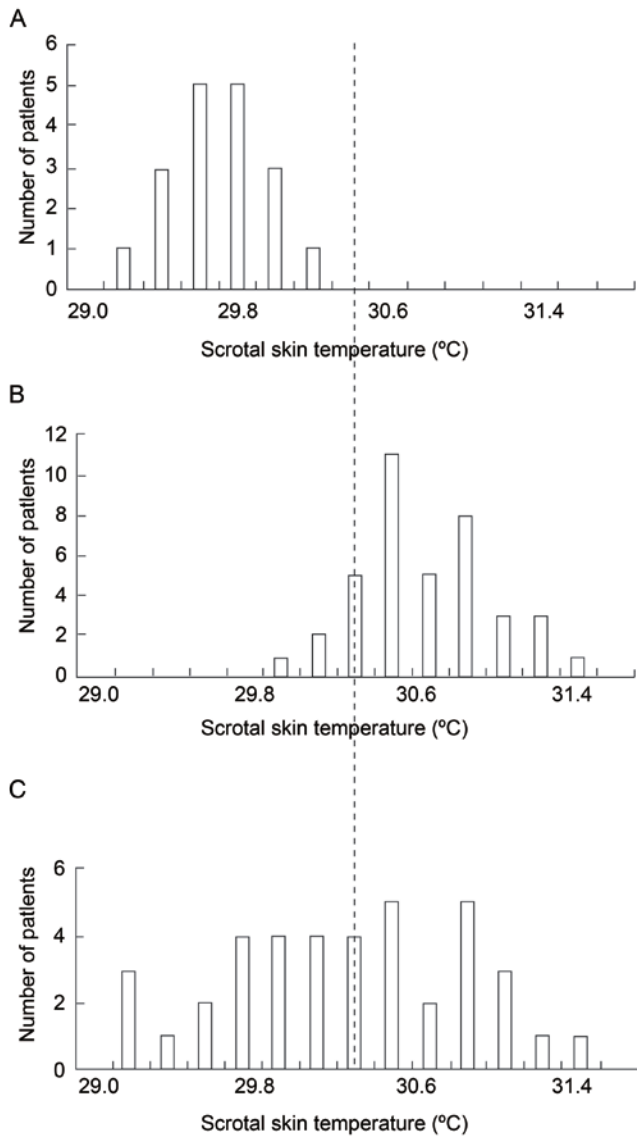


Figure 1. Scrotal skin temperature in the control and experimental groups: (A): control group; (B): experimental group before acupuncture treatment; (C): experimental group after acupuncture treatment.

#### 4 Discussion

This study confirms previous statements by other authors that the mean scrotal skin temperature in infertile men is significantly higher than that observed in fertile men [12] and that patients with low sperm output usually exhibit a scrotal temperature that is markedly higher than their body temperature [11–14, 16].

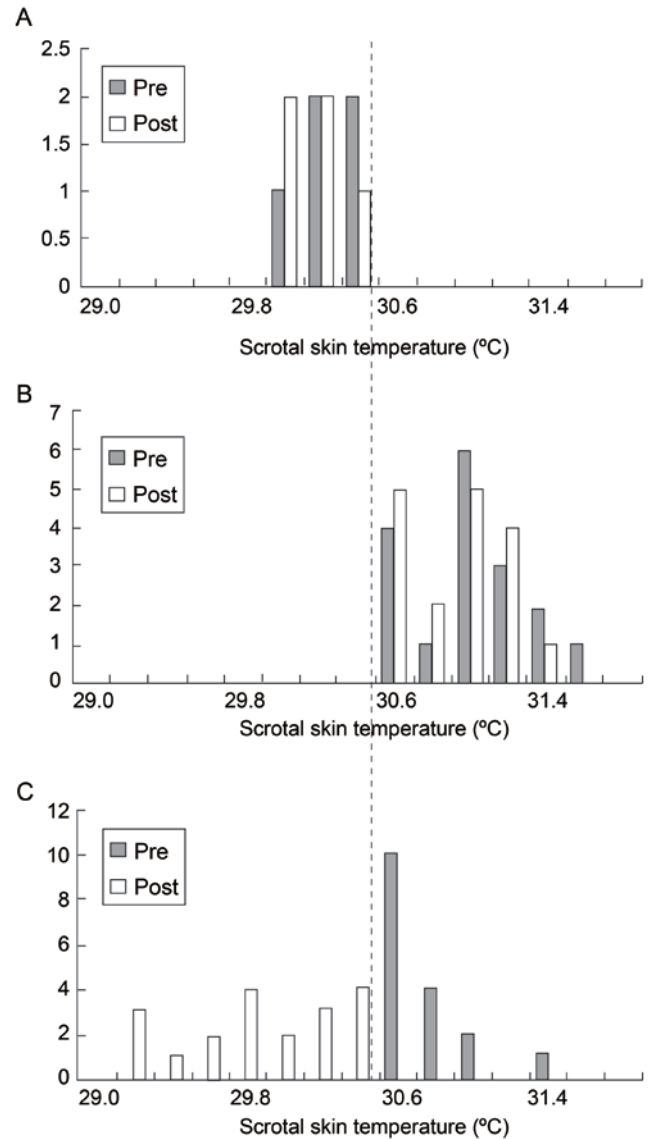


Figure 2. Scrotal skin temperature before (Pre) and after (Post) acupuncture treatment in the three subgroups: (A): subgroup A; (B): subgroup B; (C): subgroup C.

To define hyperthermia, we related the scrotal temperature to the femoral one, because it enabled us to obtain simultaneous thermographic images of the goal and the reference.

The major findings of this study were that 100% of the men with inflammation of the genital tract had scrotal hyperthermia and that the initially elevated scrotal skin temperature was normalized only in patients with this etiology, including 86.7% of the men

with isolated inflammation. These findings are in full agreement with the studies of Setchell [22, 23], who showed that, in mammals, testicular inflammation causes elevation of the testicular/scrotal temperature. Setchell [22, 23] assumed that high scrotal temperature leads to an increased metabolism and oxygen demand that cannot be met by the limited blood flow and thereby results in hypoxia, generation of reactive oxygen species (ROS), and, consequently, decreased sperm output. If this is the case, we can then assume that acupuncture treatment directed to the testis may enhance blood supply in the testicular artery [24], decrease the testicular temperature by heat exchange in the pampiniform plexus and interrupt the peroxidation process [25]. In all probability, acupuncture does not directly kill the pathogenic micro-organisms but rather stimulates the body's immune response. Indeed, existing laboratory research corroborates the systemic immunoregulatory actions of acupuncture [26]. The fact that 15 of the 17 men with normalized scrotal skin temperature had an increased sperm concentration supports this assumption.

One may argue that the men who responded to the acupuncture treatment simply experienced a coincidental increase in sperm density. However, in our earlier study [17], this possibility was excluded by using a control group of untreated patients with low sperm density.

An additional conclusion of this study fits that of Jung *et al.* [12] and Hjollund *et al.* [13], who claimed that levels of testosterone, FSH and luteinizing hormone (LH) were not associated with scrotal hyperthermia. Indeed, all five men with an isolated etiology of high FSH levels had normal scrotal temperature.

We also found that the femoral (corporal) temperature of the patients with low sperm density was statistically similar to that of the normal fertile men. Moreover, this parameter was not changed following acupuncture treatment. We conclude, therefore, that there is no relationship between corporal temperature and sperm production, and that the performed acupuncture treatment was specific only to the scrotum.

Two results of this study are unclear:

(a) One would expect that, in patients with varicocele, where there is a ROS-mediated damage to the sperm count [27, 28], acupuncture would interrupt the peroxidation process and thereby improve sperm output. In fact, the acupuncture treatment was useful for only two of the eight patients with varicocele. To verify this unsuitability, further measurement of ROS

levels before and after treatment should be performed in varicocele cases.

(b) We cannot yet explain why two mixed cases with inflammation and high FSH levels were not affected by the acupuncture treatment, whereas two others showed normalization of their scrotal skin temperature and an increase in their sperm concentration. This phenomenon may be related to the question of which of the mixed etiologies is the dominant one and will require further investigation. On the other hand, the result of the acupuncture treatment in the unsuccessful subgroup B may have been affected by the initially higher scrotal skin temperature than that in the successful subgroup C. Perhaps prolonged acupuncture treatment could be beneficial for mixed cases.

This study did not deal with the possible association between sperm quality and scrotal skin temperature. However, other authors came to a conclusion that acupuncture has a positive effect on sperm integrity [29, 30]. Our previous investigations also showed that sperm activity (including percentage of viability and motility), as well as the integrity of the axoneme, was significantly increased on treatment [31]. The question of whether this influence is associated with a decrease in scrotal temperature should be further verified.

In conclusion, low sperm count in patients with inflammation of the genital tract is associated with scrotal hyperthermia, and, consequently, these men can benefit from acupuncture treatment.

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