

Quantitative evaluation of spermatozoa ultrastructure after acupuncture treatment for idiopathic male infertility

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Objective: To evaluate the ultramorphologic sperm features of idiopathic infertile men after acupuncture therapy.

Design: Prospective controlled study.

Setting: Christian-Lauritzen-Institut, Ulm, IVF center Munich, Germany, and Department of General Biology, University of Siena, Siena, Italy.

Patient(s): Forty men with idiopathic oligospermia, asthenospermia, or teratozoospermia.

Intervention(s): Twenty eight of the patients received acupuncture twice a week over a period of 5 weeks. The samples from the treatment group were randomized with semen samples from the 12 men in the untreated control group.

Main Outcome Measure(s): Quantitative analysis by transmission electron microscopy (TEM) was used to evaluate the samples, using the mathematical formula based on submicroscopic characteristics.

Result(s): Statistical evaluation of the TEM data showed a statistically significant increase after acupuncture in the percentage and number of sperm without ultrastructural defects in the total ejaculates. A statistically significant improvement was detected in acrosome position and shape, nuclear shape, axonemal pattern and shape, and accessory fibers of sperm organelles. However, specific sperm pathologies in the form of apoptosis, immaturity, and necrosis showed no statistically significant changes between the control and treatment groups before and after treatment.

Conclusion(s): The treatment of idiopathic male infertility could benefit from employing acupuncture. A general improvement of sperm quality, specifically in the ultrastructural integrity of spermatozoa, was seen after acupuncture, although we did not identify specific sperm pathologies that could be particularly sensitive to this therapy. (Fertil Steril® 2005;84:141-7. ©2005 by American Society for Reproductive Medicine.)

Key Words: Idiopathic male infertility, sperm ultrastructure, transmission electron microscopy, acupuncture

The use of traditional or complementary/alternative medicine (CAM) for health care has been increasing (1, 2), including the use of acupuncture for the treatment of infertility. In 2002, the World Health Organization (WHO) released a global policy to assist countries in regulating traditional medicine to increase safety and effectiveness, improve standardization, protect cultural heritage, and preserve traditional knowledge.

It is estimated that about 10% of men are infertile, and that the male partner is responsible for up to 50% of infertility among couples. In 40% to 50% of males with infertility, the etiology is unknown. Research has shown that acupuncture can result in endocrine changes and relief for women with menstrual disorders (3-5). Numerous studies of acupuncture treatment on infertile men have also been conducted. Reports from uncontrolled trials using acupuncture on infertile men have shown a positive effect on sperm concentration and motility (6-8), an increase in testosterone, and some improvement in luteinizing hormone (LH) level (7, 8).

These studies have also shown an increase of normally shaped sperm and a significant decrease in the percentage of morphologically abnormal sperm (7, 9). Some studies also have shown that acupuncture did not trigger subjective behavior alterations (6, 7) or influence sexual behavior (7).

Several controlled acupuncture studies have shown a positive effect on sperm production in males with low sperm quality (10, 11). A controlled trial on men with idiopathic, normal gonadotropic oligospermia revealed that pregnancy rate or normalization of semen parameters increased significantly in 74% of patients receiving acupuncture plus clo-miphene compared with 52% of those receiving clomiphene alone (12). Another controlled trial on infertile men showed a positive effect on the 279 cases of male infertility treated

by the combination of acupuncture and Chinese herbal medicine (13). In our previous studies, the results showed that acupuncture could improve sperm quality, fertilization rate (14), and pregnancy rate (15) in assisted reproductive technology (ART).

Encouraging results prompted us to analyze the possible effect of acupuncture treatment on sperm structure. Standard semen analysis is a relatively blunt instrument for the diagnosis of male infertility, and sperm morphology at light microscopic level has been insufficiently evaluated. Thus, we considered transmission electron microscopy (TEM) to be the appropriate tool for estimating ultrastructural changes occurring in spermatozoa or in line structure of organelles involved in fertilization after acupuncture treatment (16-19).

MATERIALS AND METHODS Patients

Male patients were recruited from the couples visiting the Christian-Lauritzen-Institut, Ulm, Germany, who had been unable to initiate a pregnancy during a period of at least 2 years of unprotected sexual intercourse. All participants had thorough clinical workups that included a clinical history, physical examination, endocrinologic studies, and laboratory testing of ejaculates. The results of combined gynecologic and andrologic examinations pointed to an idiopathic male factor responsible for the infertility of all these couples. The female partners also were required to complete infertility workups, including laparoscopy and chromopertubation to verify the patency of the fallopian tubes.

The inclusion criteria were [1] male partner with idiopathic infertility for at least 2 years whose female partner had had at least two failed intrauterine insemination treatment cycles; [2] a minimum of two pathological spermograms at an interval of 6 weeks showing oligospermia, asthenospermia, and/or teratozoospermia according to WHO criteria (20); [3] normal values in a baseline endocrine evaluation that measured follicle-stimulating hormone (FSH), LH, prolactin (PRL), estradiol (E_2), and testosterone levels; [4] a minimum of 12 months without having received andrologically effective treatment; and [5] availability of the female partner's clinical fertility data.

Exclusion criteria were [1] thyroid dysfunction, adrenal disorders, hyperprolactinemia, or any pathologic hormone parameter; [2] any cause of infertility detectable after systematic physical examination and laboratory testing, including genetic testing; [3] azoospermia; [4] infectious disease or immunologic-associated disease, or presence of any major systemic disease; or [5] abnormal psychological stresses.

Study Design and Treatment Protocol

This study was a prospective, controlled trial, approved by the ethics committee of the University of Ulm, Germany. Forty patients who fulfilled the inclusion criteria were selected for the study. All of them were willing to use acupuncture, and each patient gave his written informed consent before the start of treatment in the study. The median age of patients was 33 years (range: 25 to 46 years). The experimental group consisted of 28 men who received acupuncture treatments twice a week over a period of 5 weeks. Semen samples of 12 patients with untreated idiopathic infertility, examined as the control group, were randomized with the treated idiopathic infertile men by an independent researcher using computer software. No further treatment was allowed in the study.

We used the following acupoints as main points: Guan Yuan (Ren 4), Shen Shu (UB 23, bilateral), Ci Liao (UB32, bilateral), Tai Cong (LR 3, bilateral), and Tai Xi (KI 3, bilateral). The secondary points were Zhu San Li (St 36, bilateral), Xue Hai (Sp 10, bilateral), San Yin Jiao (Sp6, bilateral), Gui Lai (St 29, bilateral), and Bai Hui (DU 20). The location of acupoints followed the international standardized location of acupoints (21).

The needles (Viva, 0.25 X 25 mm, or 0.25 X 40 mm; Helio Medical Supplies, Inc. S,an Jose, CA) were made of sterile disposable stainless steel and were inserted in acupuncture point locations to a depth of 15-25 mm, depending on the region of the body undergoing treatment. To evoke the needle sensation, or De Qi, often described as variable feelings of soreness, numbness, tingling, warmth, and/or tension, the needles were rotated to activate the muscle-nerve afferents, the A delta and possibly C fibers (22, 23). When puncturing Shen Shu (UB 23, bilateral) and Ci Liao (UB32, bilateral), the needling sensation should be transmitted to the sacral or perineum area and anterior hypogastric zone. After 10 minutes the needles were manipulated to maintain needle sensation. The needles were left in acupuncture points for 25 minutes and then removed.

Semen Collection and Analysis

Semen samples were collected by masturbation under hygienic conditions, after a period of sexual abstinence of 3 days. Two samples from each patient, one obtained the day before treatment and one after acupuncture treatment, were analyzed following standard protocols of the WHO laboratory manual (20). Semen samples were liquefied at 37°C, then the sperm count and the different motility grades were subjectively assessed using a Makler counting chamber (El-OP, Rehovoth, Israel). An aliquot of each sample was processed for examination by TEM.

Transmission Electron Microscopy

Ultramorphologic analysis of spermatozoa was assessed by TEM, performed at the Biology Section, University of Siena, Italy. Spermatozoa were fixed in cold Karnovsky fluid and maintained at 4°C for 2 hours. The fixed semen was then centrifuged at 3000 X # for 15 minutes. The pellet was washed in 0.1 M cacodylate buffer (pH 7.2) for 12 hours, postfixed in 1% buffered osmium tetroxide for 1 hour at 4°C,

dehydrated, embedded in Epon Araldite, and cut with the LKB ultramicrotome. The sections were collected on copper grids, and stained by uranyl acetate and lead citrate.

The observation and photography were made using Philips EM 301 and CM 10 TEM (Philips Scientific[^], Eindhoven, The Netherlands) at magnifications of X1 5,000 to X75,000.

One hundred sections of each sperm sample were selected randomly for observation. Ultramorphologic features of sperm were selected to evaluate the acrosome, nucleus, chromatin, axoneme, accessory fibers, and fibrous sheath according to submicroscopic characteristics and our previous experience (17-19, 24, 25). The samples from the treatment and control groups were randomized before being examined by two skilled investigators, blinded to the groups to exclude any bias.

The quantitative evaluation of the TEM data was performed by applying the mathematical formula based on the Bayesian technique proposed by Baccetti et al. (24). This formula can evaluate, by considering all statistical possibilities for defects of examined sperm, the total number of affected spermatozoa and, consequently, the sperm devoid of defects ("healthy" sperm).

Statistical Analysis

Statistical analysis was performed at the Institute of Mathematics, the University of Augsburg. After suitable transformation, one-way analysis of variance was used for data analysis. The analysis was adjusted for values obtained before treatment by including these as covariates in the model. Number of sperm, volume, and number of healthy sperm can be assumed to follow a log-normal distribution and were thus analyzed on a logarithmic scale. For percentages, the logit transformation was used before analysis, as to correspond to the variance stabilizing transformation for the binomial distribution. $P < .05$ was considered a statistically significant difference between the acupuncture group and the control group after 5 weeks of treatment. All P values reported correspond to two-sided tests for differences.

RESULTS

Semen Analysis on a Light Microscopic Level

Semen analysis of the treatment and control groups by light microscopy showed no statistically significant changes of the median number of sperm/mL ($P = .657$) and the median volume of the ejaculate ($P = .731$). The median percentage of total motility in ejaculate increased from 32% to 37% in the control group and from 44.5% to 50% in acupuncture group, a statistically significant difference between the two groups ($P = .017$).

The semen samples from the 40 patients selected for this study showed the usual structural defects observed by TEM in infertile men. The results of ultrastructural analysis were available for all 40 patients (Fig. 1).

Number and Percentage of Healthy Spermatozoa in the Total Ejaculate

The TEM data of 40 sperm samples were analyzed using the formula of Baccetti et al. (24); according to the formula, the threshold of natural fertility is 2×10^6 healthy spermatozoon in the total ejaculate. The median percentage of "healthy" spermatozoa was very low, 0.16% in the control group and 0.06% in acupuncture group, confirming the presence of male factor infertility. The median number of healthy spermatozoa, calculated in the total ejaculate, was 0.14×10^6 in the control group and 0.04×10^6 in acupuncture group.

After 10 sessions of acupuncture treatment, TEM evaluation was performed again in both groups. A statistically significant improvement was found of the percentage ($P = .012$) and the number ($P = .002$) of healthy sperm after 5 weeks of therapy. The median of percentage of healthy sperm was increased to 0.26%, and the median number of healthy sperm reached 0.2×10^6 .

Response of Organelles to Acupuncture Therapy

We used TEM and mathematical statistical analysis (24) to assess the reaction of individual organelles responsible for sperm integrity to acupuncture therapy. The ultrastructural characteristics of organelles that indicate perfect sperm functionality were analyzed before (Fig. 2a) and after (Fig. 2b) acupuncture therapy.

Acrosome in Normal Position In the control group, 65% of sperm showed the acrosome in a normal position, compared with 69.5% in the pretreatment acupuncture group. In the other cases, the acrosome was displaced and localized far from the nucleus. After the therapy, this value reached 71.5% in the control group and 77.5% in acupuncture group. The increase was statistically significant in the acupuncture group after 5 weeks of therapy ($P = .013$).

Acrosome Of Normal Shape Only about 26% of spermatozoa in the control group and 22.5% in the acupuncture group had a normal acrosomal shape before treatment. After the therapy, the median percentage of normal acrosomal shapes in the acupuncture group showed a statistically significant improvement up to 38.5% ($P < .001$).

Normal Nuclear Shape Approximately 29% of the sperm population had a normal nuclear shape in the control group and the acupuncture group before treatment. The acupuncture treatment group had a statistically significant improvement the population of normal nuclear shape, from 30% to 42.5% ($P < .001$).

Condensed Chromatin About 36% to 39% of sperm population had condensed chromatin in the control and acupuncture groups before treatment. No statistically significant change was found between the two groups after 5 weeks of treatment ($P = .506$).

Normal Axoneme Pattern Infertile men frequently show a disturbed spermatogenesis that results in axonemal patterns different from "9 + 2" configuration. The 9 + 2 pattern was present in 52% of sperm in the control group and 46.06% in the acupuncture group before treatment. After acupuncture therapy, the median percentage showed a statistically significant increase, from 46.1 % to 52.19% ($P = .005$). This value had decreased to 38.18% in the control group after 5 weeks.

Axoneme Shape Before treatment, normal axoneme shape was 67.44% in the control group and 63.64% in the acupuncture group. After acupuncture therapy, the median percentage of the normal axonemal shape showed a statistically significant increase, from 63.64% to 67.71% ($P = .022$). In control group, this value decreased to 55.85% after 5 weeks.

Normal Accessory Fibers After acupuncture treatment, the median percentage of normal accessory fibers statistically significantly increased from 34.06% to 48.53%. In the control group, this value decreased from 48.68% to 34.06% of spermatozoa after 5 weeks. The groups showed a statistically significant difference ($P = .005$).

Normal Fibrous Sheath The normal fibrous sheath was 44.41% in the control group and 33.33% in the acupuncture group before therapy. After acupuncture treatment, the median percentage of normal fibrous sheath increased to 40.59%. No statistically significant improvement could be demonstrated between the two groups, although the acupuncture group showed a tendency toward an increase after 5 weeks of treatment.

Typical Pathologies Affecting Spermatozoa in Infertile Men

The mathematical formula of Baccetti et al. (24) was applied to the TEM data of the 40 sperm samples to evaluate the probability percentage of the presence of the most common sperm pathologies.

Apoptosis Before treatment, the median percentage of apo-ptosis in ejaculated spermatozoa was 8.18% in the control group and 7.80% in the acupuncture group. After 5 weeks, the median percentage of apoptosis decreased to 6.43% in the control group and 7.15% in the acupuncture group. No statistically significant difference between the two groups was observed ($P = .863$).

Immaturity Before treatment, the percentage of immature spermatozoa was 68.23% in the control group and 71.29% in the acupuncture group. After 5 weeks, no statistically significant changes between the two groups were observed ($P = 0.146$); the percentage of immaturity in ejaculated spermatozoa was 74.11 % in the control group and 68.43% in the acupuncture group.

Necrosis Before treatment, the median percentage of necrosis in ejaculated spermatozoa was 37.28% in the control group and 36.70% in the acupuncture group. After 5 weeks, the median percentage of necrosis in ejaculated spermatozoa was 44.03% in the control group and 34.3% in the acupuncture group. There was no statistically significant difference between the two groups ($P = .072$), although there was a trend toward a decrease in the acupuncture group after 5 weeks of treatment.

DISCUSSION

Traditional Chinese medicine and acupuncture are based on ancient medical theories, but modern, scientific neurobiological perspectives have begun to evolve over the past 40 years. These new perspectives can help us to understand acupuncture effects and mechanisms, such as how the "acupuncture signal" transfers from a mechanical signal to an electric signal to a biological signal, which produces biological response. In infertility treatment, a controlled study by Siterman et al. (11) analyzed sperm density to define the most appropriate responders to acupuncture treatment. The results showed that acupuncture might be a useful, nontraumatic treatment for individuals with very poor sperm density, especially those with a history of genital tract inflammation.

Sperm morphology assessment is a valuable and stable method for predicting the *in vivo* and *in vitro* fertilizing ability of sperm. Conventional light microscopy tests cannot identify the entire variety of morphologic defects that can occur in sperm organelles, head structures (26, 27), and tail organization. Electron microscopy is currently the only tool able to analyze the ultramorphologic status of sperm cells (detecting organelles' shape, structure, and function) to determine specific sperm quality; TEM enables viewing of sperm sections and provides two-dimensional, detailed anatomic information of all subcellular structures.

Using scanning electron microscopy (SEM) and TEM, Bartoov et al. (26) evaluated the advantages of quantitative ultramorphologic sperm analysis in the diagnosis and treatment of male infertility. This methodology can successfully predict a patient's natural fertility potential by identifying the cause of infertility, and thus enable directing the patient to specific therapeutic options (10, 26).

Nevertheless, the use of TEM in andrology has been limited due to its inability to analyze data collected by observation of ultrathin sections of elongated and tortuous cells, such as spermatozoa that could appear several times in the same field. Another problem is the interdependence of submicroscopic sperm defects; for example, the probability of a spermatozoon to be morphologically normal is related to the degree of interdependence of each defect with the others. Probability analysis using a Bayesian technique solves the difficulties mentioned; the Baccetti formula (24) is a very sensitive and useful tool for assessing the relationship between sperm infrastructure, the success of different ART techniques (17-19), and the effect of FSH therapy on sperm ultrastructure to test the improvement of sperm quality (16, 25).

In the present study, submicroscopic and mathematical analysis performed before and after 5 weeks of acupuncture treatment showed a general improvement in the ultrastructural characteristics of sperm in the 28 treated patients. The median percentage and number of healthy sperm in the total ejaculate had increased. As far as the responsiveness of organelles to the therapy is concerned, the characteristics of the acrosome were shown to be sensitive to acupuncture therapy. Statistically significant improvements were seen in acrosome position and shape after 5 weeks. The nucleus was also sensitive to acupuncture therapy: nuclear shape showed statistically significant improvement, although chromatin condensation remained at the same level after therapy. The evaluation of the main structures of sperm head, acrosome, and nucleus allowed a prospective assessment of sperm penetration and fertilization ability.

Motility is a sperm function of highest relevance for reproduction, as each of the flagellar elements plays a key role in allowing spermatozoa to move effectively in a forward direction. The axoneme responded quite well to acupuncture therapy. The two characteristics of the axoneme, the classic 9+2 pattern and the shape, showed statistically significant improvement. The accessory fibers were also sensitive to the therapy, although the fibrous sheath was less affected by acupuncture treatment.

Combined with semen analysis at light microscopy level, the median percentage of progressive motility in ejaculate increased from 44.5% to 50% after acupuncture therapy. This statistically significant increase in motility was correlated with the improvement of axonemal pattern, axonemal shape, and accessory fibers. It was in agreement with the data of Siterman et al. (10), who found that the positive response to acupuncture therapy, related to improvement of total motility in ejaculate, was highly correlated with the axonemal integrity.

Our mathematical formula is able to detect the probability of the presence of pathologies affecting an ejaculate—specifically, apoptosis, immaturity, and necrosis. In spite of the statistically significant improvement of sperm quality, no statistical significance was found when the probability percentage of the presence of the main three sperm pathologies was compared before and after the therapy.

In conjunction with ART or even for reaching natural fertility potential, acupuncture treatment is a simple, noninvasive method that can improve sperm quality. Further research is needed to demonstrate what stages and times in spermatogenesis are affected by acupuncture, and how acupuncture causes the physiologic changes in spermatogenesis. Our future aim is strengthen our findings by enlarging the study group for more investigations.

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