

Acupuncture Can Increase Spermatogenic Cells in Albino Rats (*Rattus norvegicus*) Exposed by Heatstroke

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Abstract: Global warming is the newest condition that causes many re-emerging diseases. Heatstroke has been predicted as one of the global warming effects that made an important role in reproductive disease. Heatstroke with a high temperature in environmental conditions can potentially cause infertility, especially in physiological functions of the reproductive system. The objective of this study is to examine the potential of acupuncture in increasing spermatogenic cells and testosterone levels. Acupuncture therapy is expected to be an alternative solution for reducing body temperature and restoring the function of the reproductive system. In this study, 20 albino rats were used and divided into five treatments, wherein negative control group (C-) was not exposed to heatstroke and acupuncture and positive control group (C+) was exposed to heatstroke for 20 seconds. Treatment groups (T1, T2, T3) were exposed to heatstroke and acupuncture for 5; 10; and 20 seconds/rat/day. The acupuncture treatment has been performed on *Wei-Jian* and *Ming-Men* points for one week. The test was prepared with hematoxylin-eosin staining to evaluate spermatogenic cells. The blood serum collected for assay was standardized by testosterone ELISA kit. The data of spermatogenic cells and testosterone levels were analyzed using ANOVA, followed by Duncan test when there were significantly different results ($p < 0.05$). The results showed that the spermatogenic cells of (C+) were significantly different ($p < 0.05$) from those of T1, T2, and T3 and the testosterone levels of the treatment groups were not significantly different ($p > 0.05$). It can be concluded that acupuncture can increase the quality of spermatogenic cells in the testis that has been exposed to heatstroke although it has no effects on testosterone level.

1 INTRODUCTION

Heatstroke is a high-temperature condition caused by environment, heat, and direct sunstroke. Heatstroke can also be called abnormal high temperature that has a great potential of occurrence in Indonesia, considering its geographical location that has a tropical climate. This uncertain heat condition is exacerbated by the lack of ventilation and global warming (Ayo *et al.*, 2011).

Infertility problems are often encountered by adult men and if not treated immediately, will give an impact on the sterility of reproductive organs. Recent research results suggest that 8% seek medical help to treat infertility symptoms (Ashamu *et al.*, 2010). Infertility disorders are also experienced by livestock that has economic value in Indonesia, which has an impact on the decreasing quality and

quantity of livestock sector. One of the most important causes of infertility besides infectious diseases is the extreme temperatures caused by global warming that affect livestock reproduction system (Boone and Huston, 1963).

Heatstroke can happen in Indonesia. Climatographic factors in tropical regions make heatstroke conditions cause infertility cases in humans and livestock. Temperatures above 27°C can decrease the quality of spermatozoa and decrease the rate of conception in livestock (Sari, 2014).

The presence of heatstroke may trigger a decrease in the immune system of the body and activate the release of the interleukin-1 cytokine (IL-1). The release of IL-1 as a compensation of the immunosuppressed state is followed by increased body temperature and decreased food intake of each cell. IL-1 also performs a system of a blockade in

the function of gonadal glandular impulses in the hypothalamus and vice versa stimulate the adrenal gland axis. The impact of the blockade system on the gonadal glands decreases the secretion of Gonadotropin-Releasing Hormone (GnRH), which also implies the decreased production of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) (Dantzer and Kelly, 2008). Heatstroke may also increase lipid peroxidase processes that lead to increased reactive oxygen species (ROS) and become the beginning of spermatozoa cell death process (Dobson *et al.*, 2003).

Acupuncture has been developed as a traditional medicine technique in overcoming health problems. This technique can be performed by using acupuncture needles and laserpuncture. Acupuncture centers are present at acupuncture meridian points and specific points associated with a specific disease (Xie and Preast, 2007).

The *Wei-Jian* and *Ming-Men* points are important in body heat control and when stimulated, they can control blood flow, reduce heat, and organize intracellular and extracellular ion channels (Xie and Preast, 2007). Stimulation at this point can also protect the body's *chi* energy from exposure to heatstroke.

The objective of the study is to prove the potential of acupuncture to work as an alternative therapy to reduce body heat and to affect fertility improvement on quality aspects of spermatogenic cells and testosterone levels.

2 MATERIALS AND METHODS

2.1 Ethical Clearance

This study was permitted by animal care and use ethical committee of Faculty of Veterinary Medicine Universitas Airlangga with registration number No.686-KE.

2.2 Treatment

In this study, 20 albino rats were used and adapted one week before treatment. The details of treatment in experimental animals are as follows: the negative control group (C-) was not exposed to heatstroke and acupuncture, positive control group (C+) was exposed to heatstroke for 20 seconds. Treatment groups (T1, T2, T3) were exposed to heatstroke and acupuncture for 5; 10; 20 seconds/rat/day. Incubator was set to 45°C to conduct a heatstroke effect.

The acupuncture treatment used a specific needle for the meridian points. The targets of the meridian points of the reproductive system are *Wei-Jian* and *Ming-Men* points. Acupuncture treatment has been done along a week.

Albino rats were operated for a minor surgery to isolate testicle organs. Testicular histopathologic preparations were prepared in the pathology laboratory with hematoxylin-eosin staining (HE). The interpretation was conducted using Johnsen's (1970) method of five fields of view in each replication with 400 magnifications. The scoring categories are: 1) no germ cells and Sertoli cells; 2) no germ cells; 3) there are only spermatogonia; 4) there are only a few spermatocytes; 5) no spermatozoa and spermatid cells, but many spermatocytes; 6) few spermatid cells; 7) no spermatozoa, but many spermatid cells; 8) a few spermatozoa cells; 9) many spermatozoa but unorganized spermatogenesis; 10) complete spermatogenesis and tubular seminiferous.

The blood that has been taken intracardially was silenced in the test tube to obtain blood serum. Blood serum was stored in cold temperatures and prepared for an ELISA test to determine testosterone levels. Optical value density (OD) and absorbance results were analyzed.

2.3 Data Analysis

Data from each of the variables in this study were tested statistically using ANOVA test. When the results showed significant differences ($p < 0.05$) then the Duncan test continued.

3 RESULTS AND DISCUSSION

The results of testicular histopathologic readings and testosterone levels obtained were tabulated according to the data, then analyzed statistically with the SPSS v20 for windows program. The mean and standard deviation (SD) interpretations of testicular histopathology and testosterone levels are presented in **Table 1**.

Table 1: Mean and standard deviation of spermatogenic cells and testosterone level.

P	Spermatogenic cells ($x \pm SD$)	Testosterone level ($x \pm SD$)
C-	8,3 ^a ±0,416	1,6 ^a ±0,109
C+	4,2 ^d ±0,000	1,4 ^a ±0,174

T1	6,2 ^a ±0,489	1,4 ^a ±0,182
T2	6,6 ^c ±0,574	1,6 ^a ±0,246
T3	7,6 ^b ±0,526	1,6 ^a ±0,107

Different superscripts in the same column indicate significant differences among treatments ($p < 0.05$).

The results showed significantly different spermatogenic cells ($p < 0,05$) between C+ and C-, T1, T2, and T3. The treatment groups T1 and T2 were not significantly different ($p > 0.05$).

The results showed no significant difference on testosterone level ($p > 0,05$) between the treatment groups. This suggests that there was no effect of acupuncture therapy on testosterone levels of the experimental animals exposed to heatstroke.

Acupuncture therapy is expected to improve the quality of spermatozoa and can be recommended for treatment since the mass motion repair reaches 70-80%, progressive individual motion, and semi-dense concentration. Acupuncture therapy indicates the improvement and regeneration of germ cells.

Stimulation at the *Ming-Men* point can restore the temperature after receiving heatstroke exposure. Stimulation at the *Ming-Men* point can open the orifices of the skin pores, cool the blood, and open the electrolyte and homeostasis channels. The body's response to the metabolic process will return to normal with stimulation at the *Ming-Men* point, which is also a relaxation point (Xie and Preast, 2007).

Stimulation at the *Wei-Jian* point and the *Ming-Men* point, especially in the intervertebral region can improve the performance of the reproductive system. *Ming-Men* can restore the function of spermatogenesis by repairing the damaged germ cells. The meshing point that also controls the rehydration setting can cool the reproductive organs and kidneys. Indication in addition to the return of reproductive function is also a neutral cell function in suppressing metabolism after exposure to heatstroke (Xie and Preast, 2007).

Testosterone hormone is a corticosteroid derivative hormone, especially in males. Many testosterone hormones are produced by Leydig cells. Through the impulse arising from Interstitial Cell Stimulating Hormone (ICSH) produced by the anterior pituitary, ICSH will induce Leydig cells to produce testosterone. Exposure to heatstroke does not affect the anterior pituitary and suppresses the secretion of ICSH so that the production of testosterone by Leydig cells persists (Hafez, 2000).

4 CONCLUSIONS

Based on the results and discussion, it can be concluded that acupuncture therapy increases the spermatogenic cells through histopathology of the testis and has no significant effects on testosterone levels.

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