j.Egypt.net.med.Assoc 79, no 2. 426 – 441 (2019)

EFFECT OF TRADITIONAL CHINESE MEDICINE TECHNIQUES ON REPRODUCTIVE EFFICIENCY OF HEAT STRESSED RAMS

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ABSTRACT

Heat stress is an important factor that affects sperm production and viability. Rams may be completely sterile or show lower fertility during late summer as a result of the heat stress. Acupuncture has recently been used in the management of subfertility and assisted reproduction. This study was aimed to investigate the effect of some traditional Chinese medicine (TCM) techniques on sperm parameters of heat stressed rams. Twelve mature, healthy rams (14-18 months age),were divided into 3 groups randomly (4 rams in each). Group (1) served as control, Group (2) Acupuncture group, insertion of fine needles into specific points in the skin acupoints, by specific needles. Group (3) Electro acupuncture (EA) group, applying specific electric current for more stimulation to the same acupoints during treating period (three times/week for 2 months). The body weight of ram was recorded before treatment and every 2 weeks then after. Semen and blood samples were collected from all animals.Semen evaluation, testosterone hormone, cholesterol and total antioxidant were measured.

The obtained results showed a significant increase ($P<0.05$) in testosterone levels, total antioxidant in group (3) compared with group (1). While rams body weight, cholesterol levels showed non-significant difference. Regarding semen quality, results showed significant improvement ($P<0.05$) in semen volume, semen concentration, individual motility and non-significant increase in live spermatozoa in group (3) compared with Group (1) and significant decrease ($P<0.05$) in sperm abnormalities in group (3) compared with group (1). In conclusion the EA manipulation can be used for improvement of semen quality and quantity in heat stressed rams.

Key words:

Electro acupuncture, heat stress, TCM, semen quality, ram, testosterone hormone.

INTRODUCTION

Stress is a major underlying factor in infertility **(Reynolds, 2016).** Because of the delicate balance between the hypothalamus, pituitary and reproductive glands, stress can alter sperm counts, motility and cause impotence **(Mayorga-Torres *et al.*, 2017).** Heat stress is a crucial factor that affects sperm production and viability, also temporary infertility could be induced in rams as a result of overheating. According to **Nour El-Din (2013),** summer temperatures in Egypt are extremely high, reaching 38 °C to 43 °C. Based on the historical records over a period of twelve years (1999 - 2010). Temperature has a pronounced effect on the ram's semen quality. Rams may be completely sterile or show lower fertility during late summer as a result of the heat stress. Acupuncture has a history of 3000 years in China and is progressively used throughout the world, especially in the UK**, (Thomas *et al.*, 2001),** US, **(Burke *et al.*, 2006)**, Australia **(Xue *et al*., 2008)** and Japan, **(Yamashita *et al.*, 2002)** for a variety of disorders, including subfertility **(Ng *et al.*, 2008; Madaschi *et al.*, 2010; Moy *et al.*, 2011)**. Moreover, acupuncture has recently been used in the management of subfertility problems. Numerous investigations on the use of acupuncture in assisted reproduction are booming worldwide, especially in the United Kingdom, Sweden, Austria, Germany, and Denmark **(White, 2003).** These concepts are quite different from conventional Western paradigms of disease and body function. Acupuncture's effectiveness is correlated with its effects on modulating the neurophysiology and hormonal mechanisms of the body.

According to traditional Chinese veterinary medicine (TCVM), acupuncture involves the insertion of fine needles into specific points in the skin (acupoints) for balancing the flow of energy throughout pathways (meridians) in the body to achieve the homeostasis that exert the required effect. Electro acupuncture stimulation (EA) refers to applying specific electric current for more stimulation **(Pei *et al*., 2005).Chen *et al.*,(2011)** reported that, the production and quality of semen can be improved by acupuncture. Kidney, liver and spleen are the three organ systems that can be addressed with acupuncture manipulation when dealing with male infertility **(Chen *et a*l., 2011).** The ways that acupuncture increases fertility is by reducing stress and by strengthing the immune system which may play important role in enhancing reproductive **(Sherman *et al*., 1997).**

Stress induces release of cortisol hormone in the brain **(Mayorga-Torres *et al*., 2017)** with subsequent neurochemical balance alterations. **Eshkvari *et al*., (2015)** suggested that EA at stomach meridian 36 (st36) may be a viable therapeutic tool for chronic stress. **Sparrow and**

Golianu (2014) stated that, there were evidence that acupuncture decrease the stress response in both human and animals. **Pei *et al.*, (2005)** observed an improvement of sperm quality after acupuncture, specifically in the integrity of spermatozoa. **Gao *et al.*, (2012)** found that, the electro acupuncture in special acupoints improved sperm production after scrotal heat treatment. Furthermore, **Wang *et al.*, (2018)** reported that TCM can use as an alternative treatment for male infertility with poor sperm quality and as a therapeutic strategy that can improve sperm quality in infertility patients. Sperm quality is known to be an important factor in male fertility **(Cooper *et al.*,2010)**. This study was aimed to investigate whether application of TCM techniques(acupuncture and electroacupuncture)will be effective for the improvement of sperm quality of heat stressed rams or not.

MATERIAL AND METHODS

I.Animals and housing:

Twelve healthy, mature Barki rams (14-18 months age) were housed on Animal Reproduction Research Institute (ARRI) experimental farm, their body weight ranged from 38-50 Kg were used in this experiment during the period of April to August. The animals were clinically healthy, kept on a balanced ration under standard feeding and management system of the ARRI experimental farm, [Agriculture Research Center, Giza, Egypt].

The experimental rams were trained for semen collection 2 months before the start of the treatment. They were groomed, wool sheared and their body weights were recorded. Rams were divided into 3 groups (4 rams each). Group (1) served as control, Group (2) Acupuncture group, insertion of fine needles into specific points in the skin acupoints, (Baihui (Du20), Guanyuan (Ren 4), shenshu (UB 23, bilateral), taicong (Liv 3, bilateral), and zhusanli (ST 36, bilateral). Group (3) Electro acupuncture group, applying specific electric current for more stimulation to the same acupoints. The treating period was 30 minutes daily for 3 days/week, then rest 4 days. This technique was applied for 8 successive weeks for rams in groups (2) and (3).

II.Acupuncture and electroacupuncture:

Acupoints were selected for acupuncture, and electroacupuncture. Fine needles were inserted in animals of Group (2) and stimulated with electric current (EA) of Group (3).

The methodology was conducted by the method reported by **Liu *et al.*, (2009)**. Experimental rams were restrained in right recumbency, and stimulated with needles alone Group in (2) and EA in Group (3), at 40-60 Hz for 30 min with a 1mA pulse of 0.5ms duration via the

electrical current. Somatic stimulation was generated by a stimulator (JIAJIAN CMNS6-1 needle stimulator, Wuxi Jiajian Medical Instrument Co., Ltd., Xishan District, Wuxi, China).

III.Body weight:

The body weight of all rams in the experiment was recorded before treatment and every 2 weeks till the end of the experiment.

IV.Blood sampling:

Blood samples were collected from all rams by jugular vein puncture before treatment and every 2 weeks then after. Blood was collected into clean, sterilized tubes and centrifuged at 3000 rpm for 15 minutes. Serum was harvested and kept at -20 ° C until assayed for determination of serum testosterone hormone**.**

V.Semen collection and evaluation:

Semen was collected once a week using an artificial vagina of small ruminant. Collected semen samples were transferred immediately to the incubator adjusted at 37 ° C to be evaluated. All surfaces with which the semen gets in contact were kept warm at 37 ° C. Ejaculates were evaluated for:

- Volume (ml).

- Individual sperm motility (%) according to **(Evans and Maxwell, 1987).**

-Live/dead sperm ratio using eosin-nigrosine stain.

- Sperm abnormalities according to the scheme proposed by **Blom (1977)** classified into major and minor sperm defects.

- Sperm cell concentration/ml according to **Smith and Mayer, (1955).**

- Seminal plasma was obtained by centrifugation of the ejaculated sample at 3000 rpm/15 minute stored at -20 ºC for biochemical assay.

VI.Biochemical and hormonal assay:

- Hormonal assay for serum and seminal plasma testosterone hormone, using testosterone enzyme immunoassay test kit, BioCheck, Inc. 425 Eccles Avenue, South San Francisco, CA 94080. According to **Tietz *et al.* (1995).**
- Seminal plasma cholesterol, **(Allain, 1974)** and total antioxidant levels, **(Cortassa *et al.*, 2004),** were estimated using diagnostic kits from Lab. Dimension Co., Egypt.

Statistical analysis:

All results were expressed as means ± standard error (SE).The analysis of variance (ANOVA) was used to test the overall significance of differences among the means (IBM SPSS statistics, version 20), according to **Snedecore and Cochran (1989).**

RESULTS

Data presented in (Table 1) reveal that, a non-significant difference in body weight between treated and control groups.

Table (1): Rams body weight (kg) in different groups at different periods of treatment (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**42.50 ± 1.04**	**44.50 ± 0.87**	**45.00± 0.71**	**46.25 ± 0.85**	**49.50 ± 0.65**
Group (2)	**41.75 ± 2.36**	**43.50 ± 1.76**	**45.25 ± 2.06**	**47.00 ± 2.38**	**50.25 ± 2.78**
Group (3)	**43.50 ± 2.90**	**45.25 ± 3.12**	**46.00 ± 3.14**	**48.50 ± 2.72**	**51.75 ± 2.90**

***: significant at P<0.05**.

Data in (Tables 2, 3) showed that, serum testosterone level significantly increased (P < 0.05) after 1.5 and 2 months in the electro acupuncture treated group (3.25 ± 0.33 and 5.30 ± 0.72, respectively) compared with control group (1.50 ± 0.28 and 1.75 ± 0.29, respectively).

As well as seminal plasma testosterone level expressed significant elevation (P < 0.05) after 1.5 and 2 months (2.00 ± 0.27 and 2.28 ± 0.19, respectively) as compared to the control one (0.85 ± 0.13 and1.00 ± 0.13, respectively).

Table (2): Serum testosterone levels (ng/ml) in different groups of rams at different periods of treatment (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**0.88 ± 0.11**	**1.10 ± 0.24**	**1.13 ± 0.15**	**1.50 ± 0.28**	**1.75 ± 0.29**
Group (2)	**0.90 ± 0.07**	**1.03 ± 0.17**	**1.43 ± 0.29**	**1.88 ± 0.39**	**3.65 ± 0.84**
Group (3)	**0.68 ± 0.17**	**1.08 ± 0.34**	**1.63 ± 0.36**	**3.25 ± 0.33***	**5.30 ± 0.72***

***: significant at P<0.05.**

Table (3): Seminal plasma testosterone levels (ng/ml) of rams in different groups at different periods of treatment (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**0.18 ± 0.05**	**0.43 ± 0.10**	**0.55 ± 0.07**	**0.85 ± 0.13**	**1.00 ± 0.13**
Group (2)	**0.20 ± 0.04**	**0.48 ± 0.13**	**0.85 ± 0.19**	**1.65 ± 0.34**	**1.78 ± 0.35**
Group (3)	**0.15 ± 0.03**	**0.45 ± 0.10**	**1.15 ± 0.12**	**2.00 ± 0.27***	**2.28 ± 0.19***

***: significant at P<0.05.**

The results showed a significant increase (P<0.05) in the average rams semen volume (1.29 ± 0.06 ml); individual motility (81.75 ± 1.18 %) and sperm concentration/ml (5.20 ± 0.31) in the electro acupuncture treated group compared with control (0.88 ± 0.09 ml; 72.50 ± 1.66% and 3.84 ± 0.37 respectively) at the period 2 months of treatment, (Tables 4, 5 and 7).

The live dead ratio (%) showed a non-significant elevation in acupuncture treated groups compared with control, (Table 6). The results also, showed a significant decrease (P<0.05) in the sperm abnormalities in the electro acupuncture treated group compared with control, (Table 8).

Table (4): Rams Semen volume (ml) in rams in different groups at different periods of treatment (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**0.46 ± 0.07**	**0.65 ± 0.10**	**0.76 ± 0.06**	**0.83 ± 0.17**	**0.88 ±0.09**
Group (2)	**0.55 ± 0.13**	**0.63 ± 0.09**	**0.83 ± 0.09**	**0.95 ± 0.16**	**1.09 ± 0.17**
Group (3)	**0.53 ± 0.11**	**0.73 ± 0.11**	**1.10 ± 0.15**	**1.30 ± 0.20**	**1.29 ± 0.06***

***: significant at P<0.05.**

Table (5): Sperm individual motility (%) in rams in different groups at different periods of treatment (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**67.50 ± 3.23**	**68.50 ± 3.62**	**72.50 ± 3.23**	**73.75 ± 2.39**	**72.50 ± 1.66**
Group (2)	**65.00 ± 2.04**	**70.75 ± 2.18**	**75.00 ± 3.54**	**75.00 ± 2.04**	**77.50 ± 3.23**
Group (3)	**68.25 ± 3.12**	**68.75 ± 1.75**	**75.50 ± 2.10**	**79.25 ± 2.18**	**81.75 ± 1.18***

***: significant at P<0.05.**

Table (6): Sperm live dead ratio (%) in rams in different groups at different periods of treatment (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**69.75 ± 4.39**	**72.25 ± 2.29**	**76.50 ± 3.12**	**78.50 ± 3.23**	**78.75 ± 1.75**
Group (2)	**70.50 ± 3.20**	**73.50 ± 2.18**	**77.25 ± 2.63**	**79.25 ± 2.10**	**81.75 ± 3.20**
Group (3)	**70.00 ± 3.85**	**73.25 ± 2.81**	**75.25 ± 2.63**	**84.50± 2.10**	**88.00 ± 1.78**

***: significant at P<0.05.**

Table (7): Sperm cell concentration ($x10^9$/ml) in rams in different groups at different periods of treatment (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**2.01 ± 0.50**	**3.07 ± 0.73**	**3.69 ± 0.39**	**3.86 ± 0.23**	**3.84 ± 0.37**
Group (2)	**1.89 ± 0.09**	**3.06 ± 0.36**	**3.76 ± 0.23**	**4.25 ± 0.36**	**4.21 ± 0.32**
Group (3)	**2.26 ± 0.26**	**3.49 ± 0.33**	**3.76 ± 0.34**	**4.54 ± 0.33**	**5.20 ± 0.31***

***: significant at P<0.05.**

Table (8): Sperm abnormalities (%) in rams in different groups at different periods of treatment (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**11.00 ± 1.08**	**11.50 ± 1.76**	**10.25 ± 0.85**	**11.50 ± 1.32**	**12.50 ± 0.65**
Group (2)	**10.50 ± 1.04**	**10.25 ± 1.25**	**9.00 ± 0.82**	**9.25 ± 1.11**	**9.00 ± 1.23**
Group (3)	**11.25 ± 0.85**	**10.75 ± 1.25**	**9.50 ± 0.65**	**8.00 ± 0.41**	**6.75 ±0.85***

***: significant at P<0.05.**

With regards to seminal plasma cholesterol levels (mg/dl), results explained that, a non-significant difference between studied groups all over the studied period, (Table 9).

Table (9): Cholesterol (mg/dl) in rams in different groups at different periods of treatment (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**92.50 ± 4.81**	**93.75 ± 4.54**	**100.00 ± 5.40**	**101.75 ± 6.63**	**103.50 ± 5.33**
Group (2)	**94.25 ± 3.64**	**96.00 ± 4.42**	**107.75 ± 4.64**	**107.25 ± 4.33**	**104.50 ± 3.97**
Group (3)	**90.50 ± 4.29**	**94.50 ± 4.13**	**102.75 ± 7.06**	**106.50± 5.20**	**105.75 ± 4.21**

***: significant at P<0.05.**

On the other hand, seminal plasma total antioxidant (mg/dl) showed a significant elevation (P<0.05) at the period (2 months) of treatment in the electroacupuncture treated group compared with control, (Table 10).

Table (10): Total antioxidant (mg/dl) in rams in different groups at different periods (Mean ± SEM).

	Before treatment	2 weeks after treatment	1 month after treatment	1.5 months after treatment	2 months after treatment
Group (1)	**19.45 ± 0.75**	**20.06 ± 0.73**	**21.10 ± 1.19**	**20.23 ± 0.76**	**19.24 ± 0.50**
Group (2)	**19.03 ± 0.78**	**22.82 ± 0.63**	**20.29 ± 0.75**	**22.22 ± 1.05**	**21.13 ± 0.73**
Group (3)	**19.34 ± 0.81**	**20.69 ± 0.66**	**20.34 ± 1.66**	**23.29 ± 0.80**	**23.68 ± 0.48***

***: significant at P<0.05**

DISCUSSION

Acupuncture (AC), a branch of traditional Chinese medicine (TCM), has been practiced worldwide for thousands of years. Its clinical benefits have been demonstrated in both human and veterinary medicine. Acupuncture is widely employed in assisted reproduction of human beings and anecdotally reported as very effective in treating related conditions **(Young *et al.*, 2015).** Scientific trials and clinical studies have proven the efficacy of acupuncture in treating reproductive diseases in domestic animals **(Battista,1987 and Longo,2001).** Acupuncture typically refers to the use of needles alone into acupoints, and electroacupuncture stimulation (EAS) refers to applying specific electric current for more stimulation. Ram is considered the half of the herd and hence the fertility of ram is crucial for fertilization. During heat stress, testicular temperature increases, which can influence semen quality and its biochemical parameters leading to infertility problems in animals. Hence, heat stress significantly lowers conception as well as fertility rates per insemination of male and subsequently reduces male fitness **(Bhakat *et al.*, 2014)**.

In the present study, the obtained results showed a non-significant effect on body weight gain between treated and control groups, although there was slight increase in the electroacupuncture treated group compared with control. This result agreed with that reported by **Gao *et al.*, (2012)** who found non-significant differences in the body weight between electro acupuncture treated group compared with control ones in rats. In this respect, **Elmaz *et al.*, (2007)**

recorded a significant, moderately positive correlation between the serum testosterone levels and the body weight of rams. On the contrary,**Wang *et al.*,(2019)** fount that, clinical and animal studies indicates that acupuncture induces multifaceted regulation of the body weight through complex mechanisms. The difference may be due to using different accupoints, species and protocols.

The results showed significant elevation ($P<0.05$) in the serum and seminal plasma testosterone levels after 1.5 and 2 months of treatment in the electro acupuncture treated group compared with control. These results agreed with that mentioned by **Gerhard *et al.*, (1992), Ren *et al.*, (2015) and Ren *et al.*,(2016)** they found that, the electro acupuncture (EA) significantly increased serum total testosterone (TT) and free testosterone (FT) levels in male rats with partial androgen deficiency.Also, **Yang *et al.*,(2016)** detected that,EA may modulate reproductive hormone levels and the effects seem to persist for at least 12 weeks after treatment with no side effects, it may be due to stimulation or improvement of blood circulation. However, **Lue *et al.*, (1999)** observed that the serum testosterone levels as well as the weight of the androgen-responsive glands (the seminal vesicles and coagulating glands) did not change with or without electro acupuncture EA. On the other hand, **Xia *et al.*, (2017)** explained that electrical stimulation may be an effective alternative to medications to regulate the hypothamic pituitary gonadal (HPG) axis. The repeated EA on the acupoints can regulate the function of the HPG axis; enhance serum testosterone, sperm count, and gonadotropin-releasing hormone expression **(Zhaohui *et al.*, 2007)**.

The results showed a significant increase ($P<0.05$) in the average semen volume, individual motility % and sperm concentration/ml in the electro acupuncture treated group compared with control after 2 months of treatment. This result agreed with that reported by **Zhang *et al.* (2002)**, **Siterman *et al.*, (1997)**, **Dieterle, (2009) and Siterman *et al.*, (2009)** who reported a significant improvement in sperm motility, semen volume and sperm concentration. This may be due to lowering the scrotal temperature **(Siterman *et al.*, 2009);** enhancing local microcirculation, by increasing the diameter and blood flow velocity of peripheral arterioles **(Komori *et al.*,2009**) with subsequent improvement of semen production. Also, **Kishk (2008) and Sajjad *et al.*,(2007)** reported a significant correlation between testosterone with semen volume, sperm motility and sperm concentration in rams. They also reported that a high correlation between semen volume and testosterone profile in male camels **(Deen, 2008).**

The present results showed a non-significant elevation in the sperm live dead ratio (%) in

acupuncture treated groups compared with control, and a significant decrease ($P<0.05$) in the sperm abnormalities in the electroacupuncture treated group compared with control.

These results agreed with **Zhang *et al.,* (2002) and Giordano *et al.,* (2016)** who concluded that,the acupuncture treatment increased the percentage of normal spermatozoa and decreased the percentage of morphological abnormalities and it seems to produce an improvement on canine semen morphology. The underlying mechanism may be related to the changes in hormone levels, testicular blood flow as well as Sertoli cell and Leydig cell functions after EA **(Gao *et al*.,2012)**. Also, **Eshkevari *et al.,*(2015)** summarized that application of EA after initiation of stress is effective in preventing the stress-induced increases in the hypothalamus-pituitary-adrenal axis hormones, and has potent long-lasting effects. Also, Acupuncture increases the number of neurons secreting gonadoliberin **(Lee *et al.,* 2008)**. Acupuncture most probably induces the increased secretion of FSH, which stimulates the production of follistatin and inhibin. As a result, the transformation of gonocytes into spermatogonia is enhanced, as well as the differentiation of spermatocytes into spermatids.

According to some studies, low circulating testosterone concentration has its association with poor quality semen **(Schallenberger *et al*.,1991; Gulia *et al.,*2010)**. **Javed *et al.,*(2000)** reported an association between testosterone concentrations and semen quality. Others showed non-significant correlation between testosterone concentration and semen quality **(Souza *et al*., 2011; Rajak *et al.,* 2014)** or have only a low correlation with mass activity and motility **(Santos *et al.,* 2004; Souza *et al*., 2011; Mallick *et al.,* 2016).**

The result revealed a non-significant difference in the seminal plasma cholesterol levels between studied groups. These results disagreed with what reported by **LI, (2002) and Wang *et al.,* (2019)** who recorded great success of acupuncture and electroacupuncture in down regulation of the levels of triglyceride, total cholesterol in electro acupuncture treated group compared with control. Also, **LI and Zhang (2007)** reported a significant reduction in the serum total cholesterol after EA manipulation of the acupoint St40. The difference in the cholesterol values may be due to different protocol and acupoints used.

The result showed significant elevation ($P<0.05$) in the seminal plasma total antioxidant after 2 months in the electro acupuncture treated group compared with control.

This result agreed with **Ansari and Zama (2014)** who concluded that EA treatment in conjunction with conventional drug therapy enhances antioxidant and can be used to counter free radical-mediated oxidative cell injury, induced by weakness in dogs.Some studies have

been documented that acupuncture cause enhanced humoral immunity, antibody levels, and phagocytic activity **(Morgan,1996).** It may be due to enhancement of blood circulation.

In conclusion, the electro acupuncture (EA) manipulation may enhance the semen quality and quantity in heat stressed rams. However, further research needed for other animal species.

Acknowledgments:

The authors would like to thank Prof. Dr. Jinyu, Li. The professor of TCVM, Lanzhou Institute of husbandry and pharmaceutical sciences of CAAS, China for his kind support and advice on the acupuncture and electro acupuncture protocol.

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