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Low- and high-frequency transcutaneous electrical nerve stimulation have no deleterious or teratogenic effects on pregnant mice

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Abstract

Objective To evaluate the effects of application of transcutaneous electrical nerve stimulation (TENS) at low and high frequencies to the abdomens of Swiss mice throughout pregnancy.
Design Experimental animal study.
Setting Research laboratory.
Participants Thirty Swiss mice received TENS throughout pregnancy. They were divided into three groups ($n = 10$): placebo, low-frequency TENS (LF group) and high-frequency TENS (HF group).
Interventions In the placebo group, the electrodes were applied to the abdominal region without any electrical current. In the LF group, the frequency was 10 Hz, pulse duration was 200 μs and intensity started at 2 mA. In the HF group, the same parameters were applied and the frequency was 150 Hz. All stimulation protocols were applied for 20 min/day from Day 0 until Day 20.
Main outcome measures The pregnant mice were weighed on Days 0, 7, 14 and 20 to verify weekly weight gain by two-way analysis of variance. The numbers of fetuses, placentas, implantations, resorptions and major external fetal malformations on Day 20 were analysed using the Kruskal–Wallis test.
Results No significant differences were found between the placebo and TENS groups ($P > 0.05$).
Conclusion Application of low- and high-frequency TENS to the abdomens of pregnant mice did not cause any deleterious or major teratogenic effects.

Keywords: Transcutaneous electric nerve stimulation; Physical therapy modalities; Pregnancy; Fetal development; Congenital abnormalities

Introduction

Episodes of low back pain and pelvic pain during gestation are relatively common, and prevalence rates vary between 4% and 90% [1]. Ideally, non-pharmacological procedures should be used for pain control during pregnancy in order to avoid risk to the mother or fetus. These include interdisciplinary rehabilitation programmes [2], exercises [3] and manual therapy [4]. When these approaches are not effective, pharmacological interventions and other non-pharmacological therapies can be used after analysis of the potential risks to the mother and fetus [5–7].

Transcutaneous electrical nerve stimulation (TENS) is one of the most commonly used non-pharmacological interventions for pain management [8,9].

Pain suppression may occur at a peripheral level via activation of α2A receptors by noradrenaline, or μ receptors by β-endorphin. At the spinal level, TENS increases the spinal concentration of GABA, a powerful inhibitory neurotransmitter, and reduces the concentration of glutamate, an excitatory neurotransmitter involved in pain transmission. At higher cortical levels, opioid production is increased in the periaqueductal grey matter [9,10].

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In a meta-analysis, Johnson and Martinson [11] reported that TENS has a positive effect on chronic musculoskeletal pain relief. TENS has been shown to be effective in musculoskeletal pain management during pregnancy. TENS was found to be more effective for the relief of low back pain in pregnant women compared with exercises and a specific anti-inflammatory medication [12]. No side-effects were reported in mothers or newborns, although TENS was only used after 32 weeks of gestation.

The use of TENS is contraindicated in the first trimester of gestation [7] because its effects on fetal development remain unknown. In mice with uterine blood flow restriction, the use of TENS was associated with reduced number, calibre and area of placental blood vessels; lower fetal weight; and reduced placental length and volume [13]. However, an increase in placental blood flow was reported after the application of TENS in pregnant women with blood flow insufficiency, without any collateral effects to the mother or fetus [14].

Due to the lack of information on the effects of TENS in each phase of gestation, this study was undertaken to evaluate the effects of application of low- and high-frequency TENS to the abdomens of Swiss mice throughout pregnancy.

Methods

This study was approved by the Hospital das Clínicas Ethics Committee of the University of São Paulo Medicine School (Ref. No. 586/09). The animal handling and surgical procedures followed the standards of the Brazilian Society of Laboratory Animal Sciences, and current national legislation on procedures for the scientific use of animals in research (Federal Law 11, 794, 9 October 2008).

Thirty sedentary, 90-day-old Swiss mice (*Mus Musculus*, *Rodentia Muridae*) with a mean weight of 28.4 [standard deviation (SD) 1.6] g were used in this study. The animals were kept in cages (five mice per cage) at the Experimental Biotery Laboratory, School of Medicine, University of São Paulo in a controlled, clean environment [12:12 hours light/dark cycle, ventilation, mean room temperature 22.0 (SD 2.0) °C]. Food and water were supplied *ad libitum*.

Electrical stimulation was performed in 20-minute sessions on a daily basis from 0 until 20 days of gestation in all experimental groups. Day 0 was identified by the vaginal plug [15]. The length of gestation in mice ranges from 20 to 21 days.

All the animals were immobilised inside a retainer, and electrodes were attached to their abdomens. The animals were distributed at random into three groups of 10 mice: placebo (sham TENS stimulation), low-frequency TENS (LF group; frequency 10 Hz and intensity 2.0 mA) and high-frequency TENS (HF group; frequency 150 Hz and intensity 2.0 mA). Pulse duration was set at 200 μs for both groups, and sensorial intensity was increased by 1.0 mA every 5 minutes.

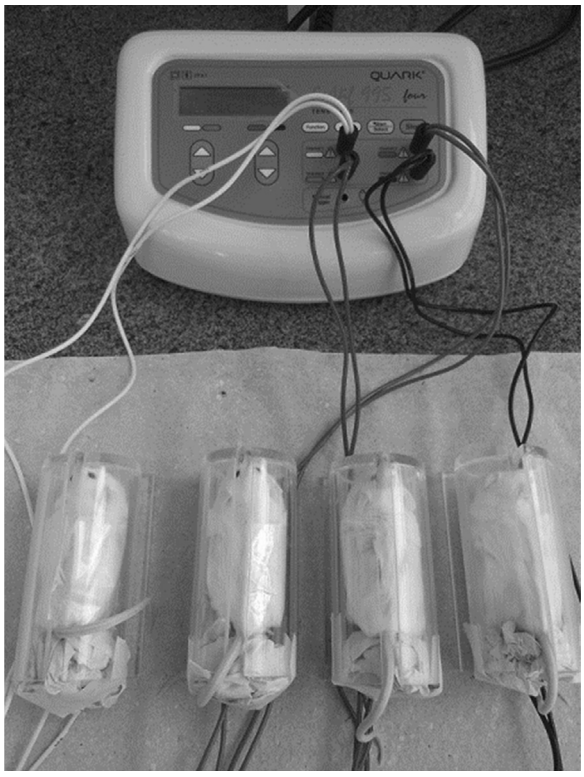

Fig. 1. Mice were immobilised in a retainer for the transcutaneous electric nerve stimulation procedure. The device had four independent channels.

TENS was applied with a symmetrical biphasic wave, using a previously calibrated Fes Vif 995 Four (Quark, Piracicaba, São Paulo, Brazil) with silicone carbon rubber 1-cm^2 electrodes attached to the abdomens of the mice in all three groups (Fig. 1). All animals were stimulated by the same researcher.

Each mouse was weighed on Days 0, 7, 14 and 20 using a CG electronic scale (Libror, Model BE_430H; CG Instrumentos Científicos Ltda., São Paulo, Brazil) with precision of 0.1 g. Weight gain was calculated by the relative change in weight gain (weight gain Δ%), defined as the difference between initial weight and weight measured at 7, 14 and 20 days of gestation [16].

The following mathematical formula was used to calculate weight Δ%:

$$\Delta\% = \frac{\text{weight on day } x - \text{initial weight} \times 100}{\text{initial weight}}$$

in which x corresponds to 7, 14 or 20.

The animals were killed at 20 days of gestation after application of anaesthetics (xylazine 0.2 mg/kg and ketamine 0.1 mg/kg). A midline abdominal incision (xiphopubic) was made immediately to expose the internal organs. Hysterectomy and hysterotomy were performed to extract and count the number of fetuses, placentas, implantations and resorptions. Finally, the myocardium was incised to proceed with euthanasia.

Fetal analysis was performed immediately after removal of the uterine horns, and a magnifying lens was used to