Int J Clin Exp Med 2018;11(5):4655-4662
www.ijcem.com /ISSN:1940-5901/IJCEM0063446

Original Article

Electromagnetic pulse radiate impairs reproductive function in young male mice

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Received August 10, 2017; Accepted February 23, 2018; Epub May 15, 2018; Published May 30, 2018

Abstract: Objective: Several studies suggest the effect of electromagnetic radiation on reproductive function in adults, but the effects during puberty are less understood. This study aimed to investigate the effects and related mechanisms of electromagnetic pulse (EMP) radiation on fertility and sex hormone concentration in prepuberal mice. Materials and methods: Three-week old male BALB/c mice were randomly allocated: experimental group received EMP radiation for 4 weeks; sham-exposure group were handled similarly while exposed to a noise simulating EMP treatment. Two weeks later, mice from both groups were mated to females to assess fertility rates. In addition, on days 1, 7, 14, 28 and 60 after irradiation, serum testosterone (T), growth hormone (GH), gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were analyzed by radioimmunoassay, and the expression of GnRH receptor (R), androgen receptor (AR) and metastasis-associated protein 1 (MTA1) in testicular tissue was assessed by PCR and immunohistochemistry. Results: Treated mice failed to impregnate females, while control mice yielded normal pregnancy rate (83.3%). Moreover, EMP radiation decreased serum GnRH, T and GH concentrations, and reduced the expression of GnRHR, AR and MTA1 in the testis. Conclusion: These findings support that EMP radiation impairs reproductive function in prepuberal mice.

Keywords: Electromagnetic pulse, BALB/c mice, reproductive endocrine, testosterone, sex hormone

Introduction

With the advent of the information era, environmental pollution with wide-spectrum electromagnetic (EM) energy or radiation has markedly increased [1]. The potential harmful effects of EM radiation particularly involve younger individuals given their frequent exposure to potential sources from early in life. An EM Pulse (EMP) is an intense burst of full spectrum EM energy [2]. EM radiation from electronic devices may have a negative impact on human health, derived mainly from non-thermal effects such as oxidative stress-induced damage to tissues and organs. Some studies suggest that certain EM radiation frequencies may have unwanted effects on reproductive endocrinology. In turn, hormonal dysregulation may lead to sexual dysfunction and infertility, miscarriage, premature birth abnormalities, and even a disruption of the offspring's sex ratio [3, 4].

Reproductive function in the mammals is regulated by a complex hormone feedback system involving the hypothalamic-pituitary-gonadal axis (HPGA) [5]. Gonadotropin-releasing hormone (GnRH) is secreted from hypothalamic neurons and transported to the pituitary portal system, reaching the anterior lobe of the pituitary where it binds to its receptor GnRHR [6, 7]. This in turn stimulates the synthesis and secretion of gonadotropins, including follicle stimulating hormone (FSH) and luteinizing hormone (LH) [8, 9]. FSH and LH exert direct effect on Sertoli and Leydig cells, respectively, to support testicular function. Binding of LH to Leydig cells promotes the secretion of Testosterone (T), an androgen essential for spermatogenesis and male reproductive function [10, 11].

Table 1. EMP on pregnancy rates, embryo counts and live offspring sex ratio (n=3)

Group	Pregnancy Rate (%)	Average of embryo count	Number of male	Number of female	Sex ratio (♂/♀)
Sham-exposure	83.33	6.7 ± 1.77	21	31	0.73 ± 0.33
EMP exposure	0	0	0	0	—

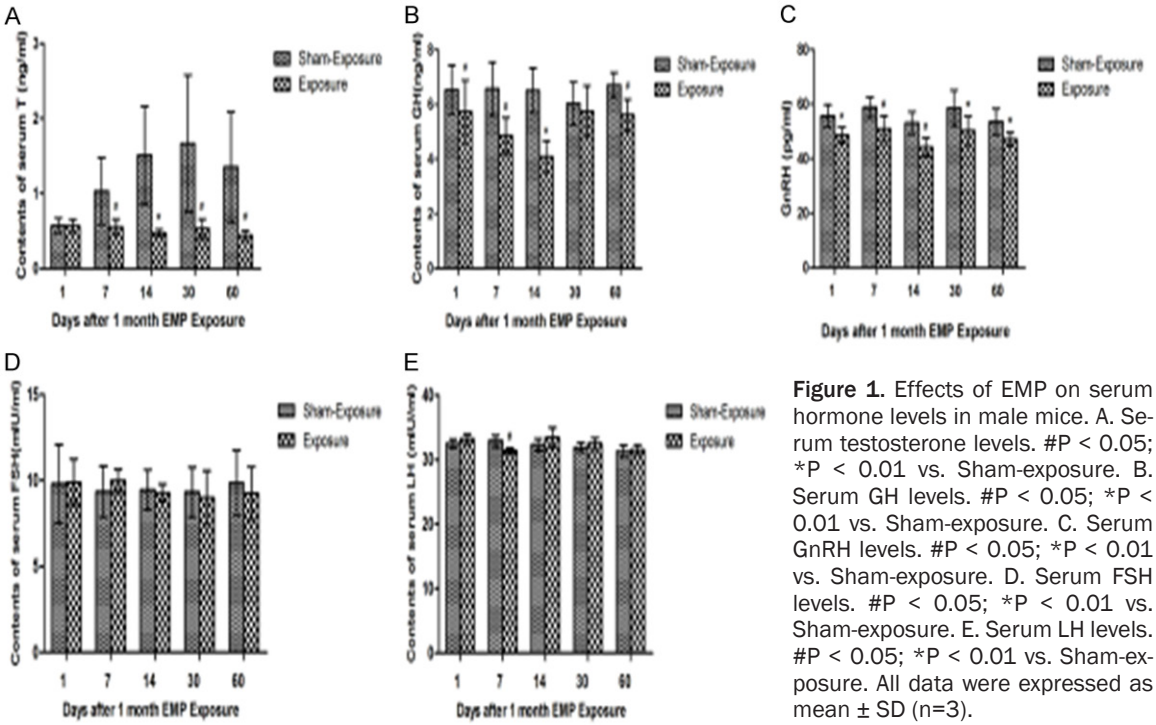

Figure 1. Effects of EMP on serum hormone levels in male mice. A. Serum testosterone levels. #P < 0.05; *P < 0.01 vs. Sham-exposure. B. Serum GH levels. #P < 0.05; *P < 0.01 vs. Sham-exposure. C. Serum GnRH levels. #P < 0.05; *P < 0.01 vs. Sham-exposure. D. Serum FSH levels. #P < 0.05; *P < 0.01 vs. Sham-exposure. E. Serum LH levels. #P < 0.05; *P < 0.01 vs. Sham-exposure. All data were expressed as mean ± SD (n=3).

Adolescence is a critical period for reproductive system development and maturation [12]. However, the effects of EM radiation exposure have not been well investigated in prepuberal animals. It was reported previously that exposure of 3-week old male BALB/c mice to EMP resulted in delayed reproductive organ development [13, 14]. However, the potential endocrine mechanisms leading to reproductive dysfunction remain elusive. Therefore, in this study we investigate the effect of EMP radiation on sex hormone levels and further explored the negative effects on reproductive function in prepubertal male mice.

Materials and methods

Instruments

Following instruments were used: EMP generator (Northwest Institute of Nuclear Technology); γ-911 Automatic RIA counter (University of Science and Technology of China Industrial Corporation); Low temperature and high-speed centrifuge (MIKRO220R, Hettich, Germany); Ultrapure water purifier (Millipore, USA); Low temperature centrifuge (Hitachi, Japan); Electrophoresis (Bio-Rad, USA); Gel image analysis system (Bio-Rad, USA).

Animals and treatment

All animal experiments were performed under approved protocols of animal use and care committee of Fourth Military Medical University. Ninety-six 3-week old BABL/c mice (9-11 g) and 32 8-week old female mice (22-24 g) were used. Male mice were randomly allocated into two groups: sham and EMPR exposure groups. Sampling was performed at 1, 7, 14, 30 and 60 d following EMP or sham exposure. Sixteen mice were used at each sampling time, except at 14 d when 32 mice were used. For treatment, mice were housed in groups of four in plastic cages with openings measuring 16 cm × 5.5 cm × 4 cm at 22-24°C. Treatment was performed 200 times per day (15:00 to 17:00 h) and 5 times per week for one month. Negative control (sham) mice were handled similarly and exposed to a sound that simulated EMP treat-

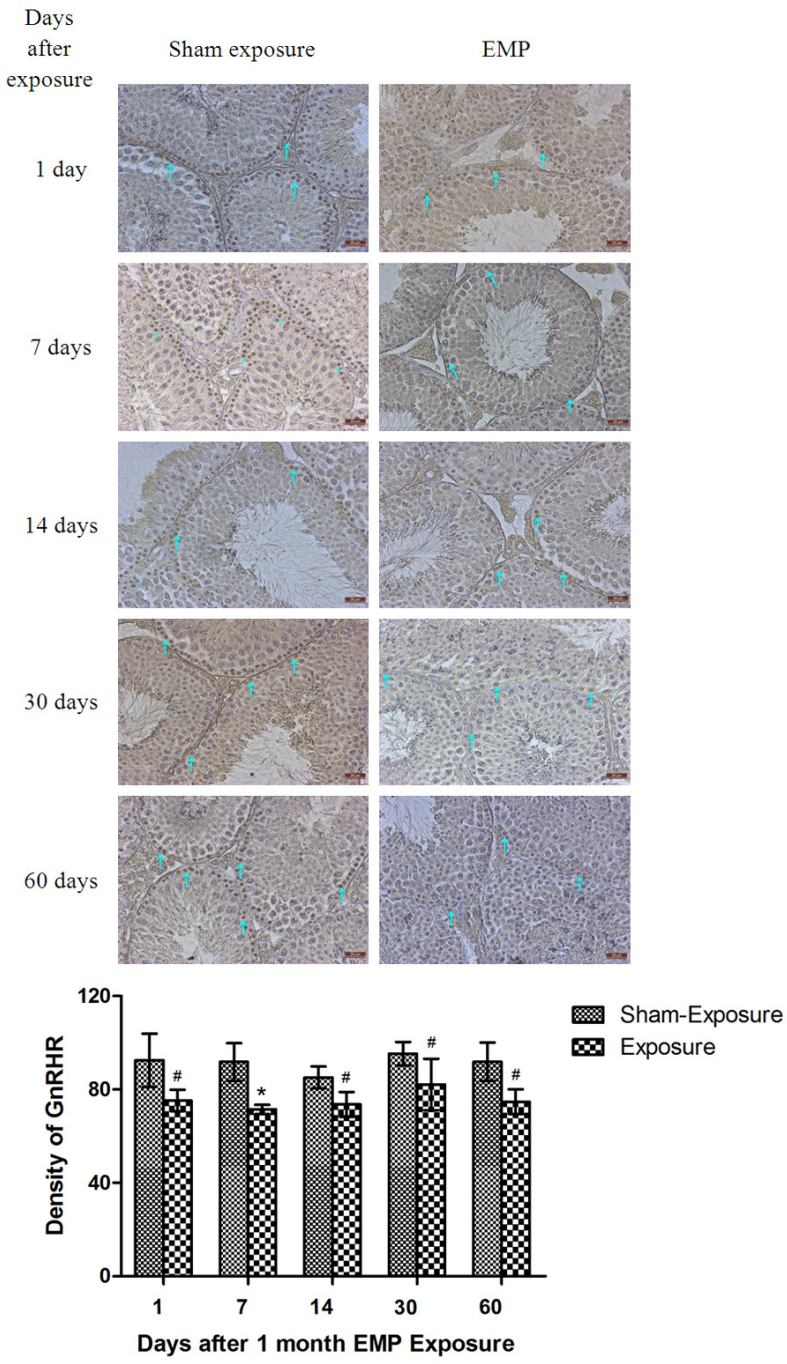

Figure 2. Effects of EMP on GnRHR expression in mice testicles. GnRHR expression was detected by immunohistochemical analysis and expressed as mean ± SD (n=3). Spermiocyte and Sertoli cells were positively stained as brown or dark-brown (indicated by the arrows). Scale bar: 50 μm. #P < 0.05; *P < 0.01 vs. Sham-exposure.

ment. All mice were weighed every four days throughout the experimental period. At each time point mice were anesthetized for blood drawing from the retroorbital sinus and immediately euthanized. The testes were then dissected from the surrounding tissues. For each mouse, one testis was plunged in liquid nitrogen and stored at -80°C, and the second was fixed in Bouin's for 24 h.

Assessment of fertility in male mice

The mice were fed to 9 weeks of age (2 weeks after radiation), the fertility of 8 male mice from each group was tested by housing each male with two females for one week. Females were checked daily for the presence of a vaginal plug. Following one-week mating period females were weighed every 4 days; a weight gain greater than 5 g was considered pregnancy in females with a plug identified. At parturition (18 days post-mating) live and still births were recorded. Two weeks after birth, the pups' sex was determined by evaluating the ano-genital distance.

Radioimmunoassay

At each time point the blood was obtained by removing the eyeballs in the mice, and centrifuged at 4,000 rpm for 15 min at 4°C to separate serum which was stored at -20°C until testing. Serum concentrations of testosterone (T), growth hormone (GH), gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH), and luteinizing hormone (LH) were determined by radioimmunoassay using the Radiation-free kit (Institute of Biotechnology, China).

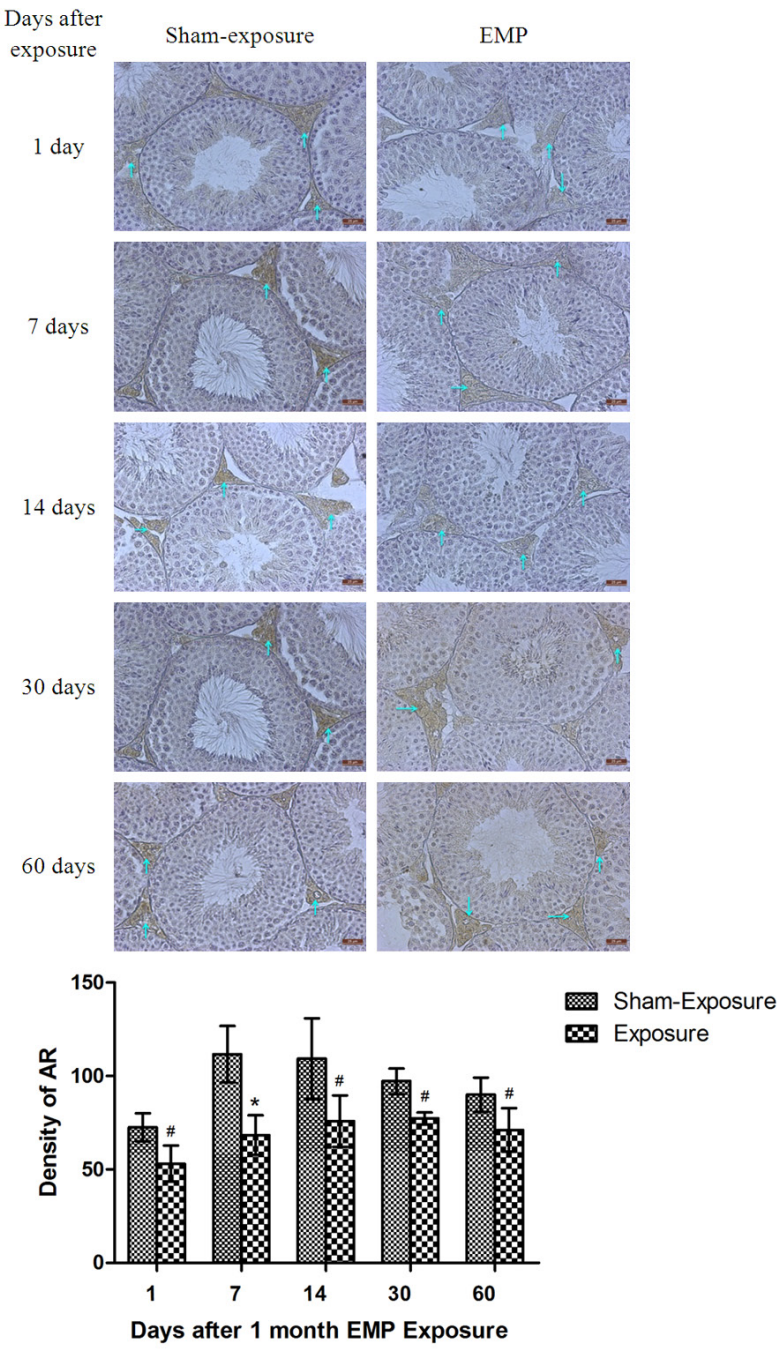

Figure 3. Effects of EMP on AR protein expression in mice testicles. AR expression was detected by immunohistochemical analysis and expressed as mean ± SD (n=3). Leydig cells were positively stained as brown or dark-brown (indicated by the arrows). #P < 0.05; *P < 0.01 vs. Sham-exposure.

Immunohistochemistry staining

Testes fixed in Bouin's solution were dehydrated in graded alcohol, embedded in paraffin, and cut into 4-μm sections for immunohistochemistry using rabbit antibodies to GnRH, androgen receptor (AR) and metastasis-associated protein 1 (MTA1), and biotin-labelled goat anti-rabbit IgG and DAB kit (Institute of Biotechnology, China).

Real-time PCR

RNA was isolated from the testes using RNAiso Plus following the manufacturer's recommendations. Total RNA was reverse transcribed using the Prime ScriptTM Kit. PCR was performed using Super Real PreMix (SYBR Green) kit and the following primers: GnRHR TAAAGATGACAGTCGCATTCG, TTCACTGGCTCTGACACC; AR (androgen receptor) CTCTTTCAAGGGAGGTTACG, CAGAGACAGAGAGGACGG; GAPDH TCCTGCACCACCAACTGCTTAG, AGTGGCAGTGATGGCATGGACT. Cycling conditions were as follows: denaturation at 95°C for 5 min; and 40 cycles consisting of 15 s denaturation at 95°C, 15 s annealing at 60°C, and 20 s extension at 72°C. $2^{-\Delta\Delta Ct}$ (ΔΔCt = experimental group ΔCt - control group ΔCt) formula was used to calculate relative expression levels.

Statistical analysis

Statistical analyses were performed using the SPSS 16.0 for Windows. The data were analysed by ANOVA. P < 0.05 was considered statistically significant.

Results

Effects of EMP on male reproductive capacity

At 9 weeks of age (two weeks after EMP exposure), 8 males from each group were housed each with 2 females for one week to monitor mating activity and outcomes. Mating with sham exposure males yielded 83.33% preg-

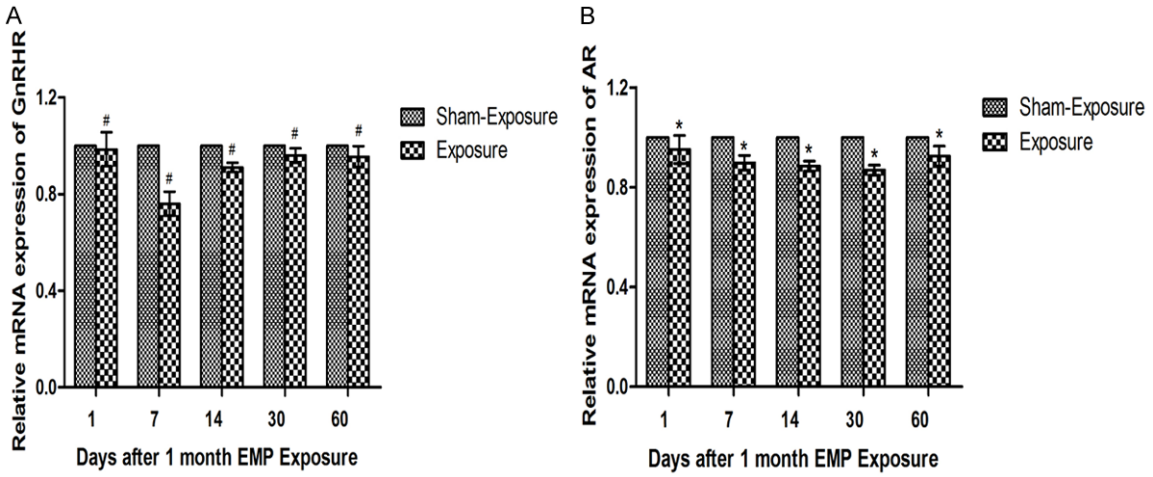

Figure 4. Effects of EMP on GnRHR and AR mRNA expression in mice testicles. GnRHR and AR mRNA expression was detected by real-time PCR and expressed as mean ± SD (n=3). #P < 0.05; *P < 0.01 vs. Sham-exposure.

nancy rates and expected birth outcomes (**Table 1**). However, all 16 females mated to EMP exposed males failed to conceive (P < 0.05).

Effects of EMP on serum hormone concentrations

As expected in prepuberal mice, a rise in testosterone concentrations was observed in control male mice from 1 to 30 days (**Figure 1A**). However, serum T concentration remained at basal levels up to 60 days following treatment in male mice exposed to EMP. Serum GH concentration remained stable throughout the experimental period in control mice (**Figure 1B**). However, in EMP exposed mice, GH concentrations decreased sharply up to 14 days following the treatment period with a moderate recovery thereafter. Serum GnRH concentration oscillated throughout the experimental period but were always lower (P < 0.05) in EMP-treated mice (**Figure 1C**). There was no significant difference in serum FSH concentration between control and EMP-treated mice (**Figure 1D**). In contrast, LH concentration was lower in EMP-treated mice compared to control mice 7 days after treatment (P < 0.05, **Figure 1E**).

Effects of EMP on GnRHR and AR expression in the testis of the mice

By immunohistochemical staining we found that both GnRHR and AR expression was lower in the testis of the mice exposed to EMP radiation at all the time points analyzed compared to control mice (P < 0.05, **Figures 2**, **3**).

Furthermore, by real-time PCR we found that both GnRHR and AR mRNA expression was lower in the testis of the mice exposed to EMP radiation at all the time points analyzed compared to control mice (P < 0.05, **Figure 4**).

Effects of EMP on MTA1 expression in the testis of the mice

To confirm that EMP impairs reproductive function of male mice, we detected the expression of MTA1, which plays an important role in the spermatogenesis in developing mouse testis [15]. By immunohistochemical staining we found that MTA1 expression was lower in the testis of the mice exposed to EMP radiation at all the time points analyzed compared to control mice (P < 0.05, **Figure 5**).

Discussion

In this study, male mice exposed to EMP during their prepuberal period failed to produce pups when they mated at 9 weeks of age, whereas control mice displayed normal fertility. Moreover, infertility in treated mice was reflected by a reduction in serum testosterone, GnRH and GH concentrations compared to sham treated mice. Previous studies have shown that electromagnetic radiation at a variety of frequencies has detrimental effects on reproductive function in adult animals [16]. In male rats the negative effects of EMP on the fertility were reflected by decreased seminiferous tubule size diameter, sperm counts and serum testosterone concentrations. Similarly, microwave irradiation at 90 W/cm^2 decreased testosterone concen-

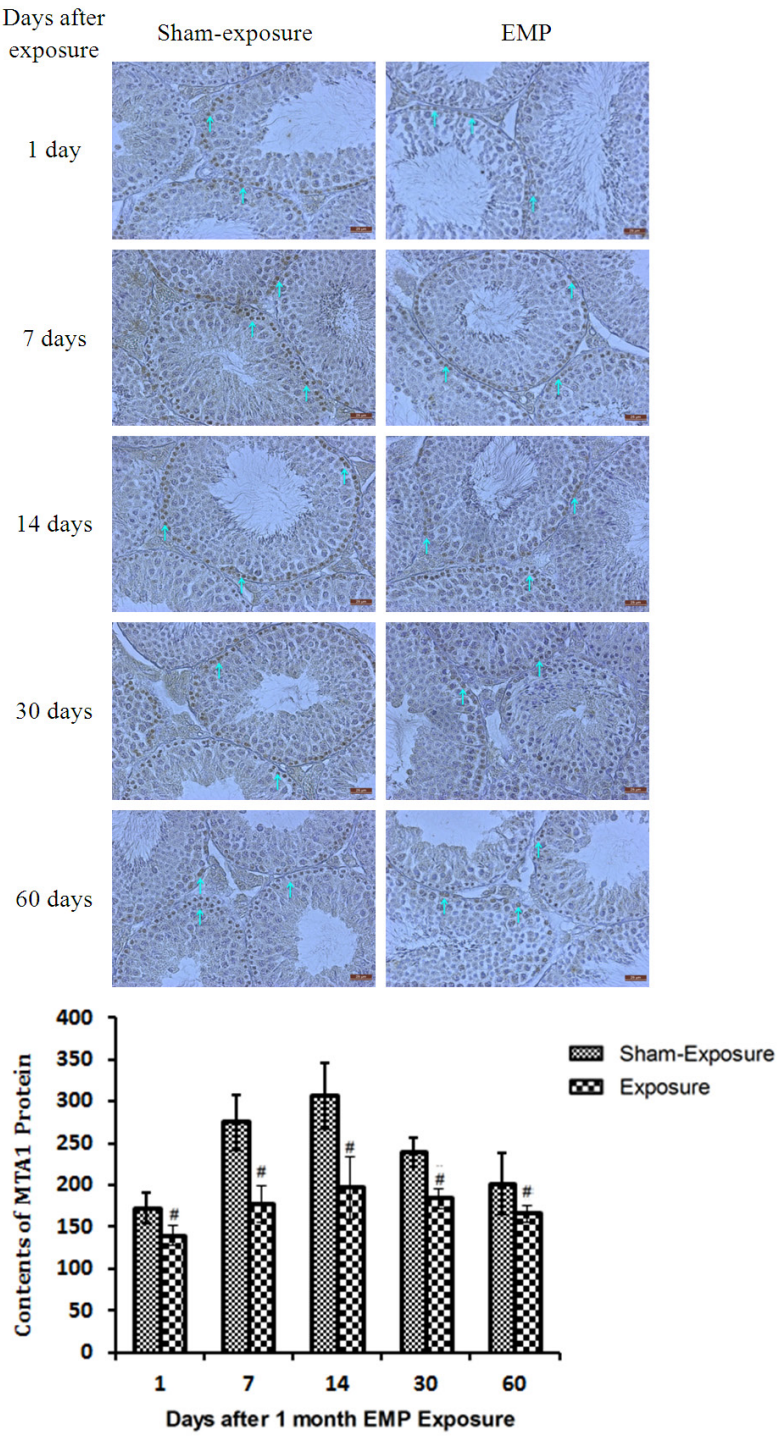

Figure 5. Effects of EMP on MTA1 expression in mice testicles. MTA1 expression was detected by immunohistochemical analysis and expressed as mean ± SD (n=3). Spermiocyte and Sertoli cells were positively stained as brown or dark-brown (indicated by the arrows). Scale bar: 50 μm. #P < 0.05 vs. Sham-exposure.

tration as well as testicular StAR and P450scc mRNA expression levels in male rats. Exposure of adult male rats to a constant magnetic field of 128 mT for 30 days also had a negative impact on serum testosterone concentration. In previous study, we observed that exposing prepuberal BALB/c male mice to EMP radiation resulted in smaller testes with concomitant reduction in sperm numbers and increase in sperm abnormalities at puberty, compared to untreated controls [14]. In present study we confirm our previous results and provide a potential endocrine explanation for the negative effects of EMP radiation on reproductive function.

Coordinated endocrine regulation of the hypothalamic, pituitary and gonadal axis is crucial for normal fertility. Therefore, we investigated the potential effects of EMP radiation on GnRH, FSH, LH, GH and testosterone concentrations in the serum. Interestingly, we found that EMP only impaired the levels of testosterone, GnRH, and GH. Reduced testosterone level observed in EMP treated mice may have potentially been resulted from a direct detrimental effect of EMP radiation on Leydig cells. Testosterone is the most important hormone in male reproduction, affecting all testicular development and spermatogenesis, as well as the development of secondary male characteristics, libido and sexual function [17]. The effects of testosterone are mediated via its interaction with the AR in target cells and then the regulation of gene transcription. It was shown that the expression of AR reached the highest levels in testicular

mesenchymal stromal cells as male rats approached sexual maturity [18]. In our study, EMP-exposed prepuberal mice showed reduced testosterone concentration accompanied by reduced expression of AR at both mRNA and protein levels, indicating delayed reproductive maturity compared to sham treated control cohort. The severe reduction in testosterone levels is consistent with the severe drop in mature sperm count observed in previous report [19]. Moreover, EMP treated mice in this study displayed sexual dysfunction, this could be also related to low androgens because of their inability to impregnate sexually mature females, unlike sham treated controls.

Exposure to EMP radiation also reduced GnRH serum concentration. In our preliminary experiments, we found that EMP radiation increased hypothalamic γ-aminobutyric acid (GABA) receptor expression. Interestingly, GABA is an inhibitory neurotransmitter, which is involved in the synthesis and release of GnRH by hypothalamic neurons. This provides a plausible explanation for decreased GnRH concentration observed in this study. However, unexpectedly, FSH and LH levels were unaffected by EMP treatment. While this inconsistency requires further investigation, we speculate that this could be a compensatory effect by pituitary neurons and/or a reflection of the reduced negative feedback stemming from reduced circulating testosterone concentrations. Consistent with the lower GnRH concentrations in EMP treated mice, we observed decreased expression of GnRHR at both mRNA and protein levels. GnRHR is a high affinity G-protein coupled receptor expressed not only in gonadotropin-secreting cells in the pituitary gland, but also in target cells in other organs such as the kidney, bladder and gonads. Since GnRH may regulate the expression of its own receptor, lower GnRH levels probably contribute to the decreased expression of GnRHR in EMP treated mice.

This prompted us to examine the concentration of GH, a single peptide chain hormone secreted by the pituitary gland that plays crucial role in bone and cartilage cell proliferation and differentiation. As anticipated, GH concentration significantly decreased in EMP-treated mice. This provides further evidence of the importance of GH not only in growth but also development of the reproductive system.

In summary, prepuberal administration of EMP radiation to BALB/c male mice resulted in sexual dysfunction and infertility. EMP induced decrease in GnRH, testosterone and GH serum concentrations and downregulation of MTA1 expression in the testis may provide a potential explanation for reproductive dysfunction. However, further studies are needed to elucidate the molecular mechanism by which EMP causes these changes. In addition, to expand the clinical significance of our findings we need confirm EMP induced infertility in young people, which will provide important guidance for the prevention of the damage of EMP on male fertility.

Acknowledgements

This work was supported by the National Basic Research Program of China (973 Program, 2011CB503704), the National Science Foundation of China (grant numbers 51677189), and Natural Science Foundation of Shaanxi Province (No. 2014JM4090, 2015JM-8454).

Disclosure of conflict of interest

None.

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