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Effects of ivermectin treatment during prepubertal and pubertal period on sexual parameters and sexual behavior in adulthood in rats

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ABSTRACT

Pediculosis is a parasitic disease that is considered a serious global public health problem. It is caused by the ectoparasite that is popularly known as lice, mainly affecting children in early childhood. The most commonly used treatment to combat this parasitosis is the macrocyclic lactone ivermectin (IVM). However, the use of IVM is contraindicated in children who are younger than 5 years old or who weigh < 15 kg because some types of drugs that are used during certain periods of brain maturation can lead to behavioral disorders. The present study evaluated the effects of IVM treatment during the prepubertal and pubertal period on sexual behavior in adulthood in male rats. Genital grooming, preputial separation, sexual behavior, sexual motivation, relative organ weight, the gonadosomatic index, and histopathology were evaluated. Oral dose of 0.2 mg/kg (therapeutic dose) of a commercial IVM formulation was administered. IVM affected genital grooming but did not influence preputial separation in prepubertal rats. Prepubertal IVM administration did not impair sexual behavior in adult rats, with the exception of the time of residence with female rats in the sexual motivation test. It did not affect relative organ weights, with the exception of the relative weight of the full seminal vesicle. It did not alter the gonadosomatic index, and no histopathological alterations were observed in different organs. These results indicate that administration of a therapeutic dose of IVM during the prepubertal and pubertal period does not alter parameters of sexual development or sexual behavior in adult male rats.

1. Introduction

Pediculosis is a parasitic disease that is considered a serious public health problem worldwide. It is caused by an ectoparasite, popularly known as lice, that lodges in the hair and mainly affects children and women (Barbosa and Pinto, 2003). Pediculosis is particularly widespread in children in school (Heukelbach et al., 2003) because children gather at education centers that favor the transmission of lice among children (Gabani et al., 2010). Macrocyclic lactones, such as ivermectin (IVM), are antiparasitic agents that are used to rid the parasites (Almeida et al., 2017).

Ivermectin is a semi-synthetic macrocyclic lactone that was first isolated from *Streptomyces avermitilis*. It is a mixture of homologues, with not < 80% of 22,23-dihydroavermectin B1a and not > 20% of 22,23-dihydroavermectin B1b. Ivermectin is the drug of choice for the treatment of pediculosis because of its broad-spectrum nematocidal, insecticidal, and acaricidal activity. It has been extensively used for the treatment of scabies and pediculosis (Gupta, 2007). Ivermectin administration at a very low dosage is recommended. The therapeutic dose is 0.2 mg/kg (Elgart and Meinking, 2003; Gupta, 2007).

In parasites, IVM acts on glutamate-controlled channels (Cully et al., 1994; Yates et al., 2003; Geary, 2005). Glutamate channels are present only in nerves and muscle cells in invertebrates. Once activated, these channels increase permeability of the cell membrane to chloride ions, thus promoting the hyperpolarization of nerves and muscle cells and resulting in paralysis and death of the parasite (Sivilotti and Nistri,

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1991).

In mammals, IVM acts by blocking the postsynaptic transmission of γ-aminobutyric acid (GABA)-mediated nerve impulses. GABA is the main inhibitory neurotransmitter in the central nervous system, the neurotransmission of which occurs through $GABA_A$, $GABA_B$, and $GABA_C$ receptors (Gupta, 2007; Sivilotti and Nistri, 1991; Bormann, 2000).

In mammals, the role of GABAergic receptors in sexual behavior has been analyzed from pharmacological studies (Agmo and Paredes, 1985). These studies suggest that intraperitoneal administration of THIP ($GABA_A$ receptor agonist) inhibited sexual behavior of male rats. On the other hand, the administration of bicuculline ($GABA_A$ receptor antagonist) had no effect on sexual behavior, but when administered concomitantly with THIP produced intense inhibitory effect on sexual behavior. When administered the $GABA_B$ receptor agonist (baclofen), it promoted almost complete inhibition of sexual behavior (Agmo and Paredes, 1985).

Other studies on the behavioral effects of macrocyclic lactone administration, such as IVM and moxidectin, also showed a high correlation of behavioral changes and the GABAergic system. Thus, Rodrigues-Alves et al. (2008) observed that moxidectin reduced sexual behavior, penile erection and hypothalamic GABA levels in adult male rats. Further studies have shown that these reductions involve interference with $GABA_A$ receptors (Rodrigues-Alves et al., 2009). The effects of IVM exposure on the sexual behavior of adult male rats were also evaluated. The authors observed that IVM was able to increase latency for the first mount and intromission, and this effect was attributed to impairment in the appetitive phase of male rats sexual behavior (Bernandi et al., 2011).

Regarding the sexual behavior of females, Moreira et al. (2014) studied the effects of IVM on female rats lordosis intensity during natural and hormone-induced estrus. The findings showed that IVM impaired the sexual behavior of female rats, regardless of estradiol modulation. More recent study showed that IVM impaired rat motor coordination due to reduced striatal levels of GABA and dopamine, as well as lowering serum testosterone levels (Moreira et al., 2017).

Thus, it is evident that there are relations between the central GABAergic system and sexual behavior of mammals. Considering that macrocyclic lactones may affect sexual behavior and that some drugs can influence behavior when they are administered during certain periods of brain maturation, investigating the possible consequences of IVM administration during critical periods of development is important. The present study investigated effects of the administration of 0.2 mg/kg IVM (therapeutic dose) during the prepubertal and pubertal period on sexual behavior in adulthood in male rats.

2. Material and methods

2.1. Animals

Healthy male Wistar rats ($n = 20$) from the Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo (FMVZ/USP), were used. Upon weaning (20 days old), the animals were housed in groups of five in polypropylene cages with a metal cover (40 cm × 50 cm × 20 cm) under controlled room temperature (22 °C ± 2 °C), humidity (45–65%), and artificial lighting (12 h/12 h light/dark cycle). Female rats ($n = 5$) were used for the sexual behavior and sexual motivation tests and were housed under the same conditions as the male rats, with controlled room temperature, humidity, and artificial lighting. The animals received free access to Nuvilab rodent chow (Nuvital, São Paulo, Brazil) and filtered water. Sterilized, residue-free wood shavings were used as bedding. All of the procedures were reviewed and approved by the Animal Care Committee of FMVZ-USP (protocol no. 4642221216) and complied with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA (1996). All efforts were made to minimize animal suffering.

2.2. Drugs and treatment

Ivermectin (6 mg Revectina tablet, Solvay Pharma, Taboão da Serra, SP, Brazil) was diluted in a water solution and administered orally by gavage at a dose of 0.2 mg/kg. The water solution alone was administered as a control (1 ml/kg).

The animals were randomly assigned to two groups: 0.2 IVM and water solution (control group). Ivermectin was administered in two doses on postnatal days 23 and 38 (i.e., 15-day interval between the first and second doses).

In humans, it is recommended to administer two doses of ivermectin (0.2 mg/kg) at intervals of 15 days for pediculosis treatment (Chosidow and Gendrel, 2016). The 15-day interval for the administration of the second dose is recommended, as ivermectin is not able to eliminate head lice eggs. It is necessary to wait for the hatching time (15 days) to avoid head lice resistance (Frankowski and Weiner, 2002). In both humans (Ottesen and Campbell, 1994; Chosidow and Gendrel, 2016) and rats (Campbell, 1989; Plumb, 2000) is recommended as a therapeutic the dose of 0.2 mg/kg IVM in the treatment of pediculosis.

Estrus was induced in female rats by a commercial preparation of estradiol valerate (1 mg Primogyna, Delpharm Lille, imported by Bayer S.A., São Paulo, SP, Brazil) diluted in saline solution and administered subcutaneously (SC) at a dose of 0.5 mg/kg (Ferri et al., 2013). All of the solutions were administered in a volume of 1.0 ml/kg.

2.3. Behavioral tests

During the prepubertal and pubertal period (Andersen, 2003; Sengupta, 2013) the animals were maintained at a regular light/dark cycle (lights on 6:00 AM to 6:00 PM). After pubertal phase the animals were maintained at the reverse dark/light cycle (lights on 10:00 PM to 10:00 AM).

Genital grooming and preputial separation were evaluated during the prepubertal and pubertal phase, and the light cycle (1:00 PM to 2:00 PM). Sexual motivation and sexual behavior were evaluated during the adulthood, and during the dark cycle (1:00 PM to 5:00 PM). In the sexual motivation and sexual behavior tests, the animals were evaluated beginning on postnatal day 90. The behavioral tests were performed under a reverse 12 h/12 h light/dark cycle.

2.3.1. Genital grooming

Genital grooming is a complex motor act that appears relatively late in ontogeny. It shows a characteristic developmental pattern around puberty and increases markedly between weaning and early post-puberty (Hernández-Arteaga et al., 2016). The assessment of genital grooming was adapted from Hernández-Arteaga et al. (2016). The rats were observed in their home cages. The occurrence of genital grooming was observed every 2 days from postnatal day 25 to postnatal day 47. The behavioral data were recorded in real time for 1 h. The observer was 0.5 m from the test cage inside the testing room. An episode of genital grooming was recorded every time one of the males licked its testicles or penis. The frequency and duration of each episode were recorded for each behavior. To ensure correct identification during simultaneous observations, the four male subjects in each cage were color-coded.

2.3.2. Preputial separation

Penile erections that occur in rats with no female present are referred to as spontaneous penile erections. They are usually characterized by extension of the engorged glans beyond the sheath and frequently coincide with or are quickly followed by genital grooming (Hernández-Arteaga et al., 2016). The assessment of preputial separation was adapted from Hernández-Arteaga et al. (2016). Preputial separation was monitored every other day beginning on postnatal day 30. Each male rat was placed on its back, and light manual pressure was applied to the posterior base of the penis, which is normally retracted

into the sheath. Total preputial separation was confirmed when the penile sheath could be retracted to fully expose the dorsal surface and approximately half of the ventral surface of the glans penis. The day of preputial separation was recorded for each animal.

2.3.3. Sexual motivation

Sexual motivation occur when a sexually inexperienced rat is exposed to a sexual incentive (receptive female) and a social incentive (male). Male rats must choose to remain with the sexual incentive (Agmo, 2014). Sexual motivation was observed in an apparatus as described by Dahlgren et al. (1991) and Moreira et al. (2017). The apparatus consisted of a circular wooden arena (80 cm diameter) that was surrounded by a 28 cm high wall. An opening (12 cm × 12 cm) in the arena wall allowed the test animals to communicate with each incentive animal cage. A wire mesh separated the incentive animal from the experimental animal, thus allowing only visual and olfactory contact. In front of these cages, the incentive zone (20 cm × 30 cm) was delineated in black on the arena floor. The arena floor was divided into three zones: male incentive zone (MIZ; delineated by black lines), female incentive zone (FIZ), and the neutral zone (NZ; defined by the remaining area of the arena). The apparatus was located in a room that was illuminated by a red incandescent light bulb.

For sexual motivation observations, incentive animals were used. A sexually receptive female was used for the sexual stimulation of experimental males (estrus was pharmacologically induced by 0.5 mg/kg estradiol valerate, SC, 24 h before the experiment began; Ferri et al., 2013), and a sexually experienced male was used for social incentive for experimental males that were located in cages outside the arena.

The sexually inexperienced experimental rats were first habituated to the testing environment in three 5 min sessions. The incentive animals were not present during habituation. Immediately before each session, the arena was cleaned with a 5% ethanol solution. The test was similar to the habituation procedure and lasted 20 min, but an incentive rat was placed in each incentive animal cage. During the test, the experimental rat could hear, see, and smell the incentive animals, but no copulatory interactions were possible.

The following parameters were recorded: time (in seconds) spent in the FIZ and MIZ and frequency of visits to each of the incentive zones (IZs; i.e., the number of times that the animal entered its four paws in the FIZ or MIZ). We also calculated the preference score, which was the ratio of the time spent in the FIZ and total time spent in both IZs: preference score = time in FIZ / (time in FIZ + time in MIZ).

2.3.4. Sexual behavior

This test was conducted in a wooden box (56 cm × 32 cm × 32 cm) that had a moveable cover and glass front with pine shavings on the floor. The test room was illuminated by two 25 W red lamps. To investigate sexual behavior, male rats were allowed to mount females that were sexually activated (i.e., in estrus that was pharmacologically induced by 0.5 mg/kg estradiol valerate, SC, 24 h before the experiment began; Ferri et al., 2013). These female lure rats were tested for receptivity before being placed with the males. Females that presented lordosis after a male mount were selected for the study. Each sexually naive male rat was individually allowed to acclimate to the behavior box for 5 min. A receptive female was then introduced to the box. Sexual behavior was assessed in 40 min time periods. The following parameters were recorded: latency to first mount (mount latency [ML]; i.e., mount without intromission), latency to first intromission (intromission latency [IL]; i.e., mount with vaginal insertion), latency to first ejaculation (ejaculation latency [EL]), number of mounts (NM), number of intromissions until the first ejaculation (NI), post-ejaculatory mount latency (post-mount latency [PML]), number of mounts post-ejaculation (NMP), number of intromissions post-ejaculation (NIP), total number of mounts (TNM), total number of intromissions (TNI), and total number of ejaculations (TNE). The sexual activity index (SAI) was calculated according to Rodrigues-Alves et al. (2008):

$$SAI = \log(1/ML \times t) + \log(1/IL \times t) + \log(1/EL \times t)\sqrt{(NM + NI) + Y}$$

where t is the time of observation, and Y is 4 when the animal's ejaculation occurred and 0 when it did not. All of the latencies were calculated in seconds.

2.3.5. Relative organ weight and gonadosomatic index

The rats were euthanized by decapitation after the behavioral tests. The organs were collected to evaluate the relative organ weight. The liver, adrenal, kidneys, testes, epididymis, ventral prostate, and seminal vesicle (full and empty, without the coagulating gland) were weighed, and the relative weight (RW) was calculated: RW = (organ weight / body weight) × 100.

The gonadosomatic index (GSI) of male rats was calculated as the proportion of the gonad mass relative to total body mass: GSI = (gonad weight / body weight) × 100 (Baber and Blake, 1991). The GSI is used to assess the sexual maturity of testis development.

2.3.6. Histopathological examination

Representative liver, adrenal, kidney, testis, epididymis, and prostate fragments were fixed in 10% formalin, dehydrated, diaphanized, and embedded in paraffin. The material was then cut into 5 μm thick sections stained with hematoxylin-eosin (HE) for histopathological analysis.

2.4. Statistical analysis

Prism 6.00 software (GraphPad, San Diego, CA, USA) was used for the statistical analysis. Student's *t*-test was used to evaluate preputial separation, sexual behavior, sexual motivation, relative organ weight, and the GSI. Before the genital grooming data evaluation, the data was normalized and then tested in the two-way analysis of variance (ANOVA) of repeated measures fallowed by a Bonferroni post hoc test. Values of $p < .05$ were considered statistically significant. The data are expressed as mean ± standard error of the mean (SEM) or as medians and their respective minimum and maximum limits.

3. Results

3.1. Genital grooming

Significant differences in genital grooming were observed between the IVM-treated group and control group (Fig. 1). The two-way ANOVA of repeated measures showed significant effects of the day of observation ($F_{7,126} = 3.826$, $p < .0008$) and interaction between factors (treatment × day of observation) ($F_{7,126} = 3.637$, $p < .0013$) on the frequency of genital grooming. The Bonferroni post hoc test showed a significant reduction of the frequency of genital grooming in the group that was treated on postnatal day 40 compared with the control group. Regarding the duration of genital grooming, the two-way ANOVA of repeated measures showed a significant effect in the day of observation ($F_{7,126} = 3.369$, $p < .0025$) and interaction between factors ($F_{7,126} = 2.804$, $p < .0095$). The Bonferroni post hoc test did not show significant differences of genital grooming in IVM-treated group and control group.

3.2. Preputial separation

Preputial separation in rats that were treated with IVM was not significantly different from the control group (Table 1).

3.3. Sexual motivation

Significant differences in sexual motivation were observed between the IVM-treated group and control group. Student's *t*-test showed

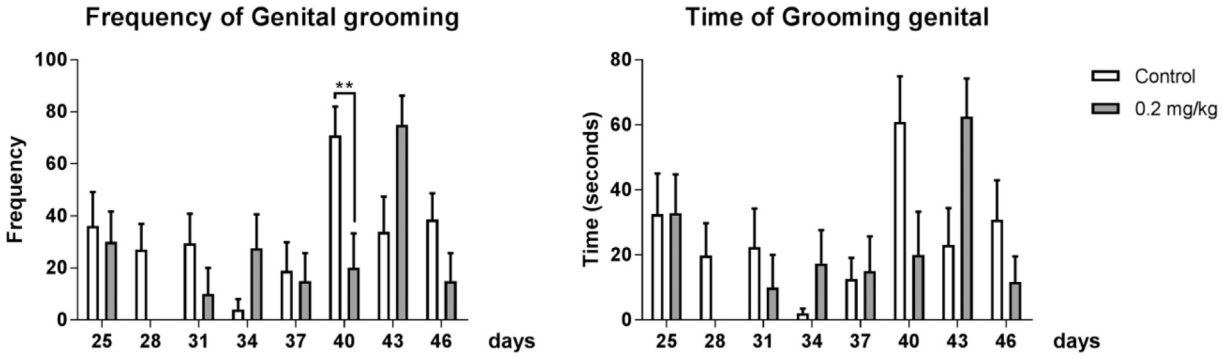

Fig. 1. Effects of ivermectin (0.2 mg/kg) and control solution (1.0 ml/kg) on the duration and frequency of genital grooming in rats that were treated during the prepubertal and pubertal period (postnatal days 23–38). The data are expressed as mean ± SEM. $n = 10$ in control group, $n = 10$ in 0.2 mg/kg ivermectin-treated group. $^{**}p < .01$, compared with control group (two-way ANOVA followed by Sidak post hoc test).

Table 1
Effects of ivermectin (0.2 mg/kg) and control solution (1.0 ml/kg) on preputial separation in rats that were treated during the prepubertal and pubertal period (postnatal days 30–38). The data are expressed as the ratio of the occurrence of preputial separation/total number of rats. *n*, number of animals per group. $p > .05$, compared with control group (Student's *t*-test).

Observation period (day)	Control	Ivermectin (mg/kg)
	($N = 10$)	0,2 ($N = 10$)
30th	1/10	2/10
32th	4/10	7/10
34th	9/10	8/10
36th	9/10	9/10
38th	10/10	10/10

significant increases in the preference score ($p = .0147$) and time in the FIZ in the treated group ($p = .0232$; Fig. 2).

3.4. Sexual behavior

No significant differences in sexual behavior were observed between the IVM-treated group and control group (data not shown).

3.5. Relative organ weight and gonadosomatic index

Relative organ weights and the GSI in adult rats were similar between groups, with the exception of the relative weight of the full seminal vesicle (Fig. 3). Student's *t*-test showed a significant increase in the relative weight of the full seminal vesicle in the treated group ($p = .0185$). Student's *t*-test did not show significant differences in the GSI between groups.

3.6. Histopathological examination

The histopathological examination of adult rats did not reveal significant changes in morphology after IVM exposure (data not shown).

4. Discussion

The present study evaluated the effects of administration of 0.2 mg/kg IVM (therapeutic dose) on sexual behavior in adult rats that were treated during the prepubertal and pubertal period. The use of IVM in

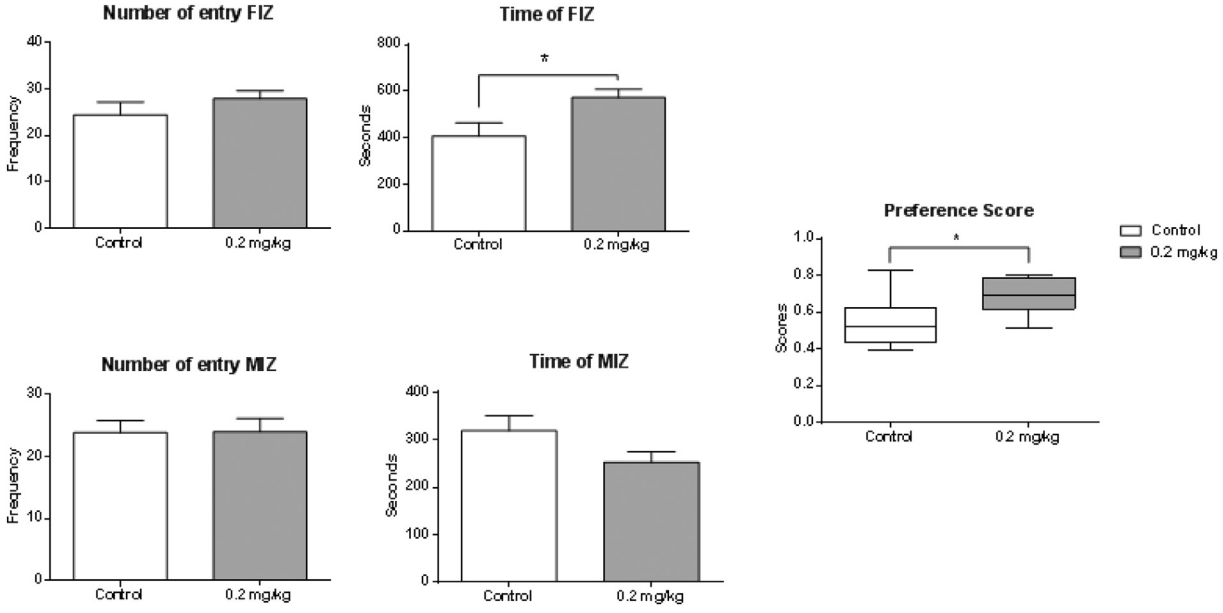

Fig. 2. Effects of ivermectin (0.2 mg/kg) and control solution (1.0 ml/kg) on sexual motivation in male rats. Sexually experienced rats were observed beginning on postnatal day 90. The data are expressed as mean ± SEM. Preference scores are reported as medians (internal full line), first and third quartiles (upper and lower box limits), and minimum and maximum values (upper and lower vertical lines). $n = 10$ in control group, $n = 10$ in 0.2 mg/kg ivermectin group. FIZ, female incentive zone; MIZ, male incentive zone. $^{*}p < .05$, compared with control group (Student's *t*-test).

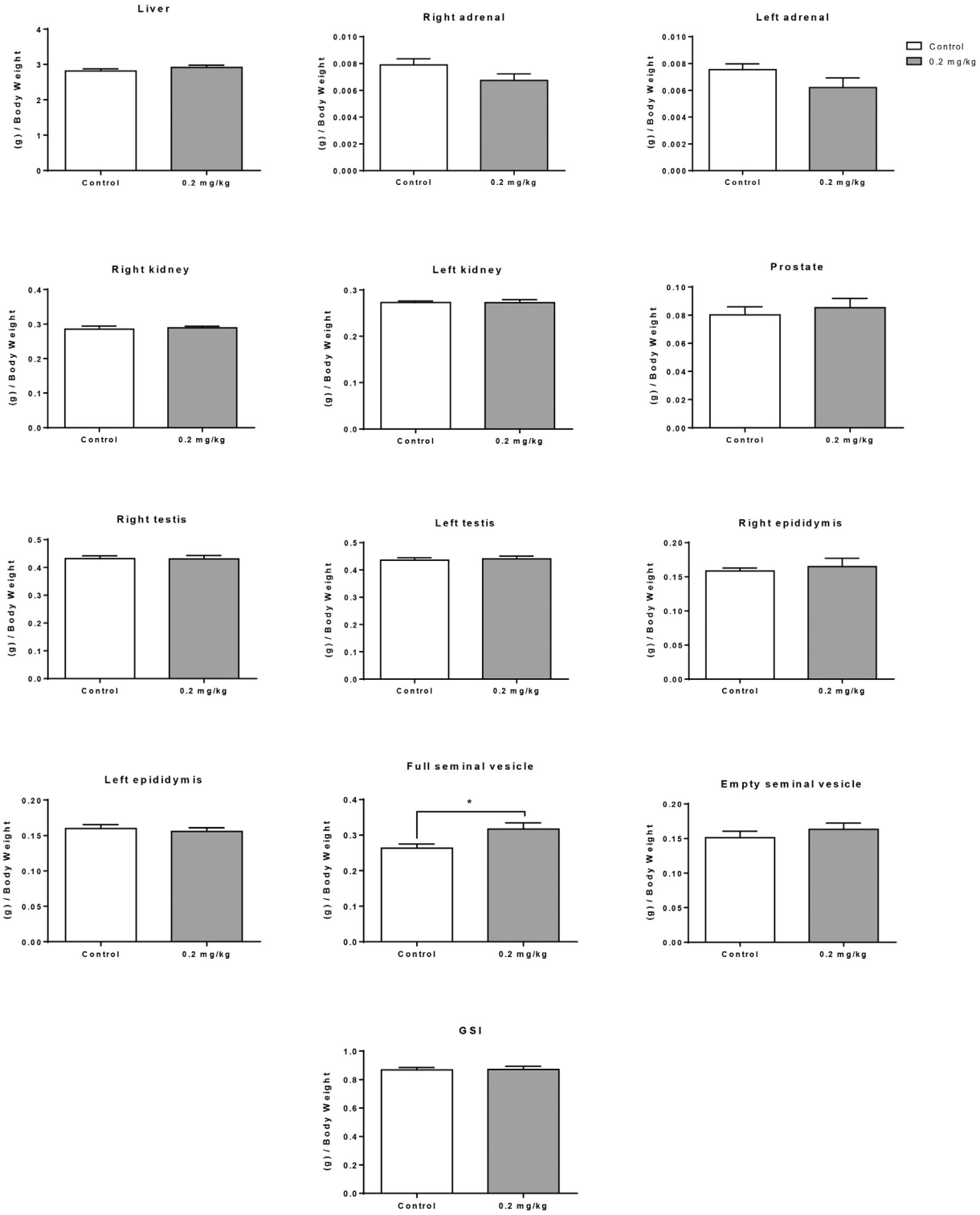

Fig. 3. Effects of ivermectin (0.2 mg/kg) and control solution (1.0 ml/kg) on relative organ weights in rats. The data are expressed as mean ± SEM. $n = 10$ in control group, $n = 10$ in 0.2 mg/kg ivermectin group. $p > .05$, compared with control group (Student's *t*-test).

children must be carefully controlled and requires medical supervision (Chosidow and Gendrel, 2016) because some drugs can cause developmental disorders and consequently alter behavior when they are administered during certain periods of brain maturation (Andersen, 2003).

Genital grooming is a behavior that corresponds to the act of cleansing that is directed toward the genital region and is associated with growth of the prostate glands and seminal vesicle in young male rats. Genital grooming is a behavior that is associated with copulatory performance in adult male rats (Hernández-González, 2000). The present results showed a significant reduction of genital grooming on postnatal day 40 in animals that were treated with IVM. According to Hernández-González (2000), male rats perform less genital grooming when the pups develop a lighter seminal vesicle and do not copulate properly, consequently altering sexual behavior. However, despite the reduction of genital grooming in rats on postnatal day 40, we did not

observe impairments in sexual behavior or relative weight of the prostate and seminal vesicle, indicating a probable reversal of the effects in adulthood.

Preputial separation is another parameter that presents a developmental pattern that is characteristic of puberty in rats. This parameter results from cornification of the epithelial lining of the foreskin that separates from the glans penis (Hernández-Arteaga et al., 2016). It is considered an early marker of puberty and a prerequisite for achieving complete ejaculation (Korenbroat et al., 1977; Hernández-Arteaga et al., 2016).

We evaluated preputial separation beginning on postnatal day 30, and all of the rats presented preputial separation between postnatal days 30 and 38. This corroborates the findings of Hernández-Arteaga et al. (2016), who found that separation of the foreskin usually occurs between postnatal days 30 and 47 in rats. Thus, administration of a therapeutic dose of IVM did not interfere with preputial separation.

Sexual behavior in male rats can be characterized as occurring in two phases: appetitive phase and consummatory phase (Agmo and Paredes, 1985). The appetitive phase is related to the animal's motivation or libido, whereas the consummatory phase is related to the performance or power of the animal to execute copulatory acts (Agmo and Paredes, 1985; Moreira et al., 2017).

The appetitive phase was evaluated to sexual motivation. In sexual motivation, the rats were exposed to a sexual incentive (i.e., a receptive female rat) and social incentive (i.e., a male rat) and were expected to remain longer with the sexual incentive (Agmo, 2014; Moreira et al., 2017). The rats that were treated with IVM exhibited a longer residence time in the FIZ compared with the control group, indicating that the IVM-treated group exhibited a greater preference for the sexual incentive than the social incentive. However, the other parameters were not different between groups, corroborating the results of Moreira et al. (2017). These data are also similar to Rodrigues-Alves et al. (2008), in which 0.2 mg/kg moxidectin was administered, SC, and no impairments in sexual motivation were observed in male rats.

Sexual behavior was evaluated to assess the consummatory phase. Rats that were treated with IVM did not exhibit alterations of the evaluated parameters. The present results corroborated Bernandi et al. (2011). These authors reported that intraperitoneal administration of 0.2 mg/kg IVM did not alter sexual behavior of inexperienced male rats 15 min after administration. Thus, administration of this therapeutic dose of IVM by different routes and with different times of administration did not impair sexual behavior in adult male rats.

In the present study, we also evaluated the relative weight of the organs, GSI, and histopathology. We found only an increase in the relative weight of the full seminal vesicle in rats that were treated with IVM. Hernández-González (2000) suggested that the weight of the seminal vesicle is related to genital grooming. Genital grooming during the developmental period in rats that corresponds to childhood in humans helps produce seminal fluid in adulthood. Thus, a better ability to perform genital grooming is associated with a greater relative weight of the full seminal vesicle. However, despite the decreases in the time and frequency of genital grooming on postnatal day 40 in IVM-treated rats, an increase in the relative weight of the full seminal vesicle was observed.

No histopathological changes were observed in the tissues that were analyzed or in the GSI. These results were similar to Moreira et al. (2017), who also did not observe alterations of the GSI or histopathological changes in rats that were treated with 0.2 mg/kg IVM. Möller (2004) administered twice the therapeutic dose of IVM (0.4 mg/kg, SC) in pregnant rats and observed no histopathological alterations in the liver, kidneys, or adrenals.

5. Conclusions

Although administration of a therapeutic dose of IVM during the prepubertal and pubertal period altered some of the parameters that were evaluated in the present study, our findings suggest that these changes did not have biologically relevant effects on sexual behavior in adult rats. We conclude that the administration of 0.2 mg/kg IVM during the prepubertal and pubertal period does not alter sexual development or sexual behavior in adult male rats.

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Declaration of Competing Interest

All authors declare that there are no conflicts of interest.

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