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Assessment of estrous cycle, ovarian and uterine tissue and fetal parameters of Wistar rats treated with Topiramate

Avaliação do ciclo estral, tecido ovariano e uterino e parâmetros fetais de ratas Wistar tratadas com Topiramato

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Abstract

Topiramate (TPM) is included in the newer generation of antiepileptic drugs and is known to have multiple mechanisms of action. The drug has also been used for reducing body weight. Its effect on reproductive tissues and estrous cycle deserve greater attention. Then, this study aimed to investigate possible effects of the drug on ovarian and uterine tissues, estrous cycle and some fetal parameters of non-epileptic Wistar rats. In Experiment I, females received tap water (C - Control group; n=8) or Topiramate (TPM group; 100 mg/kg; n=8), orally for 6 weeks. The estrous cycle and food consumption were monitored. Ovarian and uterine sections were examined under light microscopy. In Experiment II, pregnant rats of C and TPM groups received treatments during the pre-implantation, implantation or organogenesis period. In females of Experiment I, TPM had no effect on the food consumption, final body weight, weekly body weight and estrous cycle. Ovarian and uterine weight was similar in both groups. The kinetics of folliculogenesis was unaffected by treatment with the drug. There was a significant ($p<0.05$) decrease in endometrial thickness of TPM-group. In Experiment II, fetal weight was decreased ($p<0.05$) in all periods of TPM exposure. There was no effect of treatment on fetal external morphology. In conclusion, the findings indicate that TPM promotes discrete alterations in the uterine tissue, and causes decrease on the fetus weight after exposure in different gestational periods.

Keyword: Antiepileptic drug. Folliculogenesis. Endometrium. Outcome pregnant.

Resumo

O Topiramato está incluído na mais nova geração de drogas antiepilépticas e é conhecido que apresenta múltiplos mecanismos de ação. A droga também é usada como redutor de massa corporal. Seu efeito nos tecidos reprodutivos e no ciclo estral merece maior atenção. Então, este estudo teve como objetivo investigar os possíveis efeitos da droga sobre os tecidos ovariano e uterino, ciclo estral e alguns parâmetros fetais de ratas Wistar não epilépticas. No Experimento I, as fêmeas receberam água de torneira (C – grupo controle; n=8) ou Topiramato (grupo TPM; 100 mg/kg; n=8), oralmente por 6 semanas. Foram monitorados o ciclo estral e o consumo de ração. As secções ovarianas e uterinas foram examinadas em microscopia de luz. No Experimento II, ratas prenhes dos grupos C e TPM receberam os tratamentos durante os períodos de pré-implantação, implantação ou organogênese. Nas fêmeas do Experimento I, o TPM não teve efeito sobre o consumo de alimento, peso corpóreo final, peso corpóreo semanal e ciclo estral. O peso ovariano e uterino foi similar em ambos os grupos. A cinética da foliculogênese não foi

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afetada pelo tratamento com a droga. Houve uma significativa ($p<0,05$) redução na espessura endometrial do grupo TPM. No Experimento II, o peso fetal foi reduzido ($p<0,05$) em todos os períodos de exposição ao TPM. Não houve efeito do tratamento sobre a morfologia externa fetal. Concluindo, os resultados indicam que o TPM promove discretas alterações no tecido uterino e causa decréscimo no peso fetal após a exposição em diferentes períodos gestacionais.

Palavras chave: Droga antiepiléptica. Foliculogênese. Endométrio. Produtos da gestação.

Introduction

Topiramate (TPM) is included in the newer generation of antiepileptic drugs and is known to have multiple mechanisms of action (LIN, 2011). The drug is prescribed to patients whose epilepsy was not responding to traditional drugs (HERNÁNDEZ-DÍAZ et al., 2012). The biochemical and pharmacologic effects may determine its broad range of nervous system activities including anticonvulsant, analgesic, and mood stabilizing properties (RICHARD et al., 2000). The drug exerts its antiepileptic activity by blocking sodium and calcium channels, thus increasing the action potential of gamma aminobutyric acid (GABA) and inhibiting carbonic anhydrase.

In recent decades, a beneficial effect of TPM has been reported in the production of weight loss in obese patients (BRAY et al., 2003; SHAPIRA; GOLDSMITH; MCELROY, 2000). However, the pathogenic mechanisms of TPM-induced weight loss are still not completely known (VERROTI et al., 2011b). According to the authors, these mechanisms suggested by animal studies include reduced energetic efficiency, hypothalamic involvement, neuropeptides and insulin-sensitizing effects. Human studies suggest the role of reduced caloric intake, hormonal involvement and changes in glucose and lipid metabolism. It is reported that the TPM is more efficient than other drugs such as sibutramine and Orlistat in the treatment of body weight loss, but promotes several side effects including paresthesia, impaired concentration, mood problems, fatigue and difficulty in memorizing (ASTRUP; TOUBRO, 2004). The scientific results are still very conflicting as to the effect of TPM in reducing body weight (VERROTI et al., 2011b).

It is documented that women with epilepsy have a higher prevalence of reproductive disorders, including menstrual irregularities, premature menopause and polycystic ovarian syndrome (PENNEL, 2009).

The side effects promoted by TPM for pregnant women is a constant concern, since those women with epilepsy often need to continue the use of drug during pregnancy for seizure control (FRENCH, GAZZOLA, 2011; HERNÁNDEZ-DÍAZ et al., 2012). Teratogenic effects and risk of congenital malformations promoted by TPM are widely reported in literature. Studies in animals or humans reported that TPM causes skeletal anomalies (FADEL et al., 2012), pathological neurotoxic effect in the cerebral cortex (HASHIH, 2014), decrease in birth weight and increase in rate of spontaneous abortion (ORNOY et al., 2008), and hypospadias (BRAGA et al., 2013). According to various authors (FOUNTAIN, 2009; HERNÁNDEZ-DÍAZ et al., 2012; TENNIS et al., 2015), an association of TPM with an increase in incidence of oral clefts or cardiac malformations still needs further investigation.

Although TPM showed beneficial effects as an appetite inhibitor and has proven efficacious in the treatment of epilepsy, little attention even has been directed toward the effects of the drug on reproduction, particularly regarding the effects in ovarian and uterine tissues, as well as in the estrous cycle. Thus, this study investigated the effects of TPM in the estrous cycle and histological structure of ovaries and uterus of non-epileptic Wistar rats, and the effects of drug

on the some fetal parameters, when the treatment occurs in different gestation periods.

Materials and Methods

Animals

Female Wistar rats, 70 days old, weighing approximately 220 g were obtained from the Univ. Estadual Paulista (UNESP- Botucatu, SP, Brazil) and kept at the Faculty of Sciences and Letters (UNESP-Assis, SP, Brazil). The females were housed in polypropylene cages (43cm x 30cm x 15cm) with laboratory-grade pine shavings as bedding, maintained inside a ventilated bookcase (Insight Equipment Ltda., Ribeirão Preto, SP, Brazil), with controlled air exhaustion and luminosity (air exchange every hour; 12h light/dark photoperiod, respectively). The room temperature remained constant (22-24°C). The animals were fed a commercial diet (Nuvital™) and tap water ad libitum.

The experimental protocol followed the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation. The protocols were approved by the Committee for Ethics in Animal Use (Permit number: 003/2012-CEUA).

Experimental Groups

The study was delineated to comprise two experiments. In Experiment I, the females were divided into two groups (n= 8/group): Topiramate (TPM, treated with Topamax™ - Janssen-Cilag Lab., São Paulo, Brazil; at a dosage of 100 mg/kg body weight, diluted in 1.0 mL of tap water, through oral route - gavage), and Control (C, treated with equivalent volume of tap water, through oral route). The dose used in this study was chosen according to previous studies (OTOOM et al., 2004; KHOURI, 2005) and represents 19.5 times the beginning titration dose of 50 mg/day in humans, using the formula of equivalence proposed by Reagan-Shaw et al. (2007). The females received the treatments daily (8:00 hours a.m.), for six consecutive weeks. The first dose was administered at the estrus phase of the sexual cycle. After the experimental period, the rats were euthanized for examination of ovarian and uterine tissue.

In Experiment II, females not previously treated (n=24/group) were kept for mating in the evening, with a non-treated male. On the morning after mating, presence of sperm in vaginal smears indicated pregnancy and this was designated as gestation day 1 (GD). Each group, Control and Topiramate, received the treatments in different gestation periods: day 1 to 4 (n=8/group), 5 to 7 (n=8/group) and 7 to 14 (n=8/group), corresponding to pre-implantation, implantation and organogenesis periods (CARMO; PETER; GUERRA, 2004), respectively. On GD19, the females were euthanized for assessment of maternal and fetal parameters.

Estrous Cycle

In the females of Experiment I the estrous cycle was monitored daily during the treatment period by cytology of vaginal smears. The phases of the cycle were identified as proestrus, consisting of basal nucleated cells; estrus, with predominance of keratinized enucleate cells; metaestrus, consisting of leukocytes in combination with keratinized cells; diestrus, with a predominance of leukocytes.

The number of estrus cycles during the treatment period and the duration of the estrous cycle (days of an estrus to other estrus phase), were obtained for each group.

Body Weight and Food Consumption

In Experiment I, the females were weighed once a week and food consumption was recorded daily. The dams of Experiment II were weighed on GD1, GD7, GD14 and GD19.

Euthanasia of Females

At the end of the six-week period (Experiment I), the females were weighed and euthanized at the estrus phase of the estrous cycle, by means of an overdose of anesthetic (Thiopentax™, Cristalia, São Paulo, Brazil), intraperitoneally. The ovaries, uterus and vital organs of interest in the toxicological evaluation (liver, heart and kidneys) were removed, weighed and their absolute (g) and relative weights (g/100g body weight) were calculated.

On GD19, the females of Experiment II were anesthetized with ketamine (40 mg/kg; Dopalen™, Ceva Lab., São Paulo, Brazil) and xylazine (20 mg/kg; Dorcipec™, Vallée Lab., São Paulo, Brazil), by intramuscular administration, and the gravid uterine horns and ovaries were collected and examined.

Tissue Preparation

The ovaries and uterine horns were fixed in Bouin's solution, dehydrated in ethanol, clarified in xylene and embedded in Paraplast (Labware-Oxford, St. Louis, MO, USA). The blocks were sectioned at a thickness of 5-µm, in a RM2125 LEICA microtome (Germany). The sections were stained with Hematoxylin and Eosin (HE) and Mallory's Trichromic for histopathological and morphometrical analyses.

Ovarian and Uterine Analysis

The identification of ovarian follicles was based on the classification proposed by Pedersen and Peters (1968), according to Plowchalk, Smith e Mattison (1993). In ovarian sections (n=10/female/group), the atretic follicles were counted according to the classification of the degree of regression (OSMAN, 1985) as follows: a) Stage IA: in which overall shrinkage of the granulosa wall is apparent at a low magnification and detailed degenerative changes are visible at higher magnification in scattered small areas; b) Stage IB: in which the whole granulosa wall is affected by degeneration, and many nuclear fragments can be observed at the periphery of the antrum; c) Stage IIA: in which the degenerative oocyte is still surrounded by an envelope of degenerating cumulus cells or their remnants; d) Stage IIB: in which the oocyte is found "naked" in the antrum, the granulosa wall usually has a distinct inner lining and macrophages are usually present in the antrum. The number of corpora lutea was quantified and the area of corpora lutea was measured. The mean diameter of each healthy follicle was obtained for the identification of follicular type in following classes (HIRSHFIELD, 1991): a) Class I: small preantral follicles (<90 µm); b) Class II: large preantral follicles (91-260 µm); c) Class III: small antral follicles (261-350 µm); d) Class IV: antral follicles of mean size (351-430 µm); e) Class V: large antral follicles (431-490 µm); f) Class VI: mature follicles or Graaphian (>491 µm).

In the uterine cross-sections were observed the histological aspects of perimetrium, myometrium and endometrium. In the medial portion of uterus (n=5/animal/group), the following parameters were measured in morphometric analysis: height of the luminal and glandular epithelium, and thickness of the endometrium (considering luminal epithelium and stroma), myometrium (considering inner and outer layers) and perimetrium (considering epithelial and connective tissue). These results were expressed in micrometers (µm).

The morphometric analyzes of the ovaries and uterus were performed by using a digital photomicroscope (Zeiss Scope A1-Axio connected to an AxioCam ICc3 camera), and the digitalized images were obtained by the software Axio Vision, version 4.7.2.

Maternal and Fetal Parameters

In the pregnant rats of Experiment II, the gravid uterine horns and ovaries were examined. The number of corpora lutea, implants, fetuses, resorptions and placentae were obtained. Pre-implantation loss (number of corpora lutea - number of implantations / number of corpora lutea

x 100) and post-implantation loss rates (number of implantations - number of fetuses / number of implantations x 100) were calculated.

The fetuses were examined according to external morphology, under stereomicroscope (Citoval 2, Carl Zeiss, Germany).

Statistical Analysis

The data were analyzed through parametric analysis of variance (ANOVA) complemented by the Student's *t* test, when presented normality, and the results were expressed as mean ± standard deviation (SD). In the absence of normality, the data were analyzed by the nonparametric Kruskal-Wallis test complemented by Mann-Whitney, and the results were expressed as median ± interquartile deviation. Statistical analysis was conducted on GraphPad Prism software, version 5.00. Statistical significance was set at $p<0.05$.

Results

Experiment I

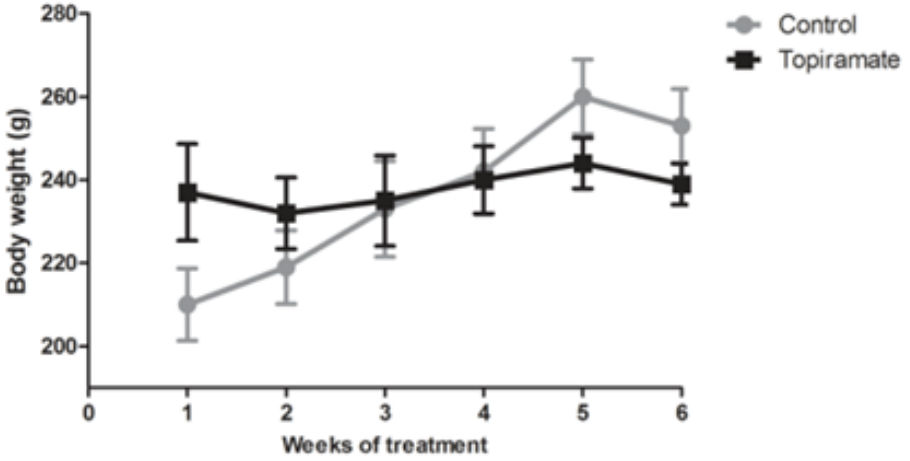
Table 1 shows that the food consumption, final body weight and absolute and relative weights of the ovaries, uterus and heart were similar ($p>0.05$) in the Topiramate group (TPM) and Control group (C). Liver and kidney weights increased ($p<0.05$) in the TPM group. Weekly body weight was similar in both experimental groups starting from the 2nd week of treatment (Figure 1).

Table 1. Food consumption, final body weight and reproductive and non-reproductive organs weight in the Control (C) and Topiramate (TPM) groups of Experiment I.

Parameters	Experimental groups (n= 8/group)	
	C	TPM
Food consumption (g/day/female)[a]	19.3 ± 1.4	15.7 ± 0.7
Final body weight (g)[a]	253.0 ± 25.1	239.5 ± 4.9
Ovaries		
Absolute weight (g)[b]	0.13 ± 0.01	0.16 ± 0.05
Relative weight (g/100g)[b]	0.05 ± 0.001	0.06 ± 0.002
Uterus		
Absolute weight (g)[b]	0.48 ± 0.10	0.53 ± 0.06
Relative weight (g/100g)[b]	0.19 ± 0.0006	0.21 ± 0.0002
Liver		
Absolute weight (g)[b]	9.26 ± 1.30	10.08 ± 1.09
Relative weight (g/100g)[b]	3.48 ± 0.0018	3.94 ± 0.0043*
Heart		
Absolute weight (g)[b]	0.87 ± 0.10	1.05 ± 0.10
Relative weight (g/100g)[b]	0.40 ± 0.0005	0.40 ± 0.0007
Kidneys		
Absolute weight (g)[a]	1.74 ± 0.19	2.12 ± 0.21*
Relative weight (g/100g)[a]	0.67 ± 0.05	0.84 ± 0.09*

*Statistical significance compared to the Control group, $p<0.05$. [a]Values are expressed as mean ± SD. Student T-test.
[b]Values are expressed as median ± interquartile deviation. Mann-Whitney test.
Fonte: Authors

Figure 1 - Weekly body weight during treatment period in the female rats of Control and Topiramate groups. Data are express as mean ± SD.



Fonte: Authors

No significant change was observed in the duration of estrous cycle or number of estrus cycles in TPM-treated females, compared to untreated animals (Table 2).

Table 2 - Estrous cycle duration and number of estrus phase during treatment period in the Control (C) and Topiramate (TPM) groups of Experiment I.

Parameters	Experimental groups (n= 8/group)	
	C	TPM
Estrous cycle duration (days)	6.0 ± 0.9	7.0 ± 2.7
Estrus phase (number)	10.4 ± 1.8	10.5 ± 2.6

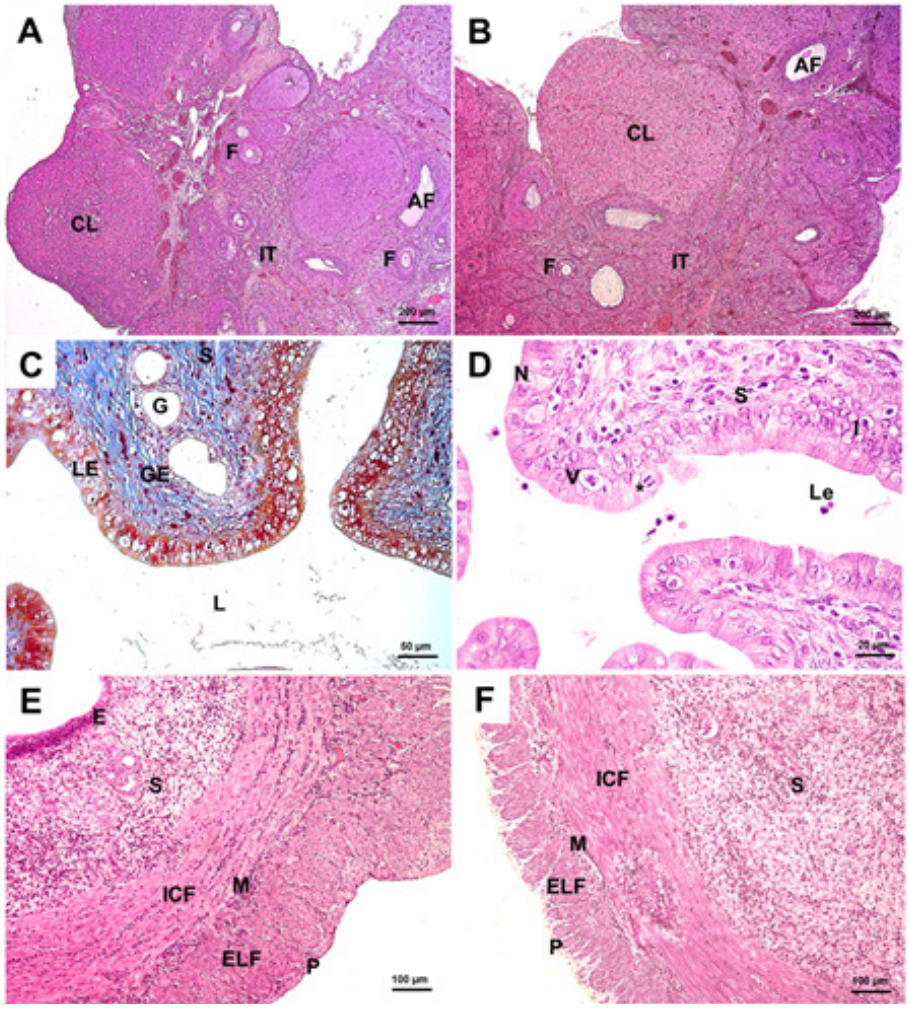
No significant difference between the groups, $p>0.05$. Values are expressed as mean ±SD. Student T-test.
Fonte: Authors

The ovarian histological sections showed that both groups, C and TPM (Figures 2A and 2B, respectively), presented corpora lutea, healthy follicles at various stages of development and atretic follicles, as well as interstitial tissue dispersed by stroma. Interestingly, the area of corpora lutea was higher ($p<0.05$) in the TPM group, when compared to the C group (Table 3). Nevertheless, the number of corpora lutea was similar ($p>0.05$) in both groups (Table 3). Figure 3A shows that there was no significant difference ($p>0.05$) between groups in the number of healthy follicles in each class of development. Figure 3B shows the number of atretic follicles, at different regression stages, in the two experimental groups. Follicles in more advanced degeneration (stage IIB) were predominant in both groups. No significant difference ($p>0.05$) was observed in the number of atretic follicles in stages IA, IB, IIA or IIB between the experimental groups.

In the uterine tissue, C group (Figures 2C, 2E) exhibited columnar luminal epithelium with many secretory vacuoles along its extension. Leukocytes were dispersing in the stroma or near the epithelium.

Little secretion was present in the uterine cavity and glandular lumen. TPM group (Figures 2D, 1F) presented discrete changes in the endometrium. Scarce secretors vacuoles and cells in mitosis were observed in luminal and glandular epithelium while there was a significant decrease ($p<0.05$) in endometrium thickness (Table 4), compared to the C group. Both the groups presented myometrium and perimetrium with similar histomorphometric characteristics (Figures 2D, 2F; Table 4). Luminal and glandular epithelium height was equal ($p>0.05$) in the two experimental groups (Table 4). Luminal secretion was represented mainly by polymorphonuclear leukocytes.

Figure 2 - Photomicrographs of ovaries (A, B) and uterus (C-F) of female rats in Control (A, C, E) and Topiramate (B, D, F) groups. In both groups the ovaries exhibit corpora lutea (CL), developing follicles (F), atretic follicles (AF) and interstitial tissue (IT). Observe in uterus of Control group (in C), the presence of many secretors vacuoles in the luminal (LE) and glandular (GE) epithelium, glands (G) in endometrial stroma (S) and scarce amount of secretion in the lumen (L). Group TPM (in D) presents luminal epithelial lining with rare dividing cells (asterisk) and evident nucleolus (N), and few secretors vacuoles (V). Leukocytes (Le) are visible in stroma (S) or uterine cavity. In E and F, observe the morphological similarity of the endometrium, myometrium (M) and perimetrium (P) in the two experimental groups. E = luminal epithelium; S = stroma; M = myometrium; ELF = extern longitudinal muscle layer; ICF = inner circular muscle layer. Hematoxylin-eosin (A, B, D-F); Mallory's Trichromic (C). Bars= 200 µm (A, B); 100 µm (E, F); 50 µm (C); 20 µm (D).



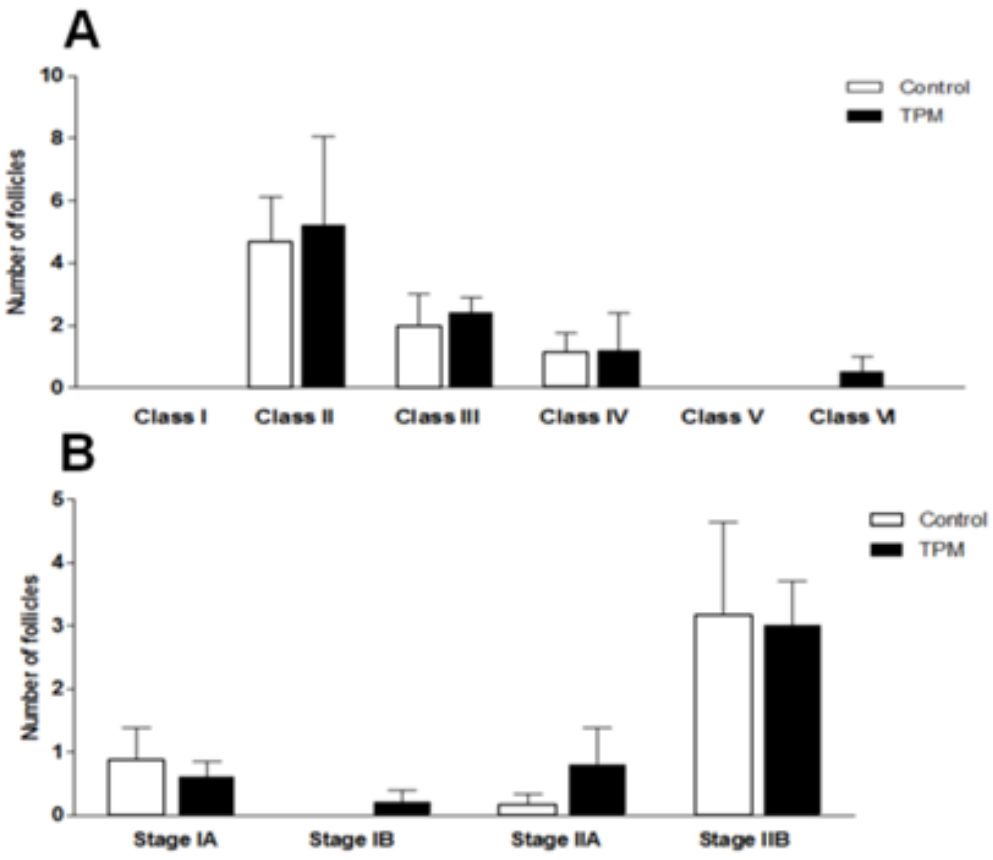
Fonte: Authors

Table 3 - Area and number of corpora lutea in the Control (C) and Topiramate (TPM) groups of Experiment I.

Parameters	Experimental groups (n=8/group)	
	C	TPM
Corpora lutea area (mm^2)	448.9 ± 184.8	572.8 ± 184.7*
Corpora lutea number	10 ± 4.8	10 ± 3.8

*Statistical significance compared to the Control group, $p<0.05$. Values are expressed as mean ±SD. Student T-test.
Fonte: Authors

Figure 3 - Quantitation of healthy follicles in each class (in A), and follicular quantification in different stages of atresia (in B), in the Control (C) and Topiramate (TPM) groups. No statistical difference between the groups, $p>0.05$. Mann-Whitney test. Values are express as median ± interquartile deviation.



Fonte: Authors

Table 4 - Uterine histomorphometry in the Control (C) and Topiramate (TPM) groups of Experiment I (data are expressed as μm).

Parameters	Experimental groups (n= 8/group)	
	C	TPM
Luminal epithelium height[b]	31.3 ± 9.38	34.8 ± 12.0
Glandular epithelium height[b]	14.7 ± 3.5	14.7 ± 4.2
Endometrium thickness[b]	344.4 ± 109.2	249.3 ± 81.5*
Myometrium thickness[a]	376.0 ± 212.5	411.4 ± 99.0
Perimetrium thickness[a]	20.8 ± 12.8	20.6 ± 6.5

*Statistical significance compared to the Control group, $p<0.05$. [a]Values are expressed as mean ± SD. Student T-test. [b]Values are expressed as median ± interquartile deviation. Mann-Whitney test.
Fonte: Authors

Experiment II

The maternal and fetal parameters after Topiramate treatment in pre-implantation (GD1 to GD4), implantation (GD5 to GD7) and organogenesis (GD7 to GD14) periods are shown in Tables 5, 6 and 7, respectively. No significant difference ($p>0.05$) was observed between the experimental groups in the body weight of dams, gravid uterus weight, placentae weight, litter size or pre- and post-implantation loss rates. There were no gross external morphological defects in fetuses of dams given the drug in different pregnancy periods. However, fetal weight was significantly decreased ($p<0.05$) in the TPM-treated group in comparison to the C group, in all development periods.

Table 5 - Maternal and fetal parameters in the Control (C) and Topiramate (TPM) groups, with treatment during the pre-implantation period (GD1-GD4; Experiment II).

Parameters	Experimental groups (n=8/group)	
	C	TPM
Body weight of dams (g)[a]		
DG1	244.0 ± 24.0	263.0 ± 27.5
DG7	262.5 ± 22.7	276.0 ± 39.0
DG14	300.8 ± 29.2	311.1 ± 20.9
DG19	340.5 ± 32.9	359.6 ± 26.8
Gravid uterus weight (g)[a]	43.1 ± 4.0	46.1 ± 9.8
Placentae weight (g)[b]	5.6 ± 0.8	5.9 ± 1.4
Litter size[b]	12.5 ± 1.8	15.5 ± 2.6
Fetal weight (g)[b]	1.8 ± 0.4	1.6 ± 0.3*
Pre- implantation loss (%)[a]	0 ± 2.7	0.8 ± 5.6
Post- implantation loss (%)[a]	0 ± 3.3	0 ± 3.4

*Statistical significance compared to the Control group, $p<0.05$. [a]Values are expressed as mean ± SD. Student T-test. [b]Values are expressed as median ± interquartile deviation. Mann-Whitney test.
Fonte: Authors

Table 6 - Maternal and fetal parameters in the Control and Topiramate groups, with treatment during the implantation period (GD5-GD7; Experiment II).

Parameters	Experimental groups (n=8/group)	
	C	TPM
Body weight of dams (g)[a]		
DG1	247.7 ± 24.0	264.2 ± 27.6
DG7	264.7 ± 24.9	282.5 ± 37.0
DG14	293.3 ± 24.7	303.5 ± 52.1
DG19	331.7 ± 22.4	339.2 ± 66.9
Gravid uterus weight (g)[a]	42.1 ± 6.4	36.2 ± 21.9
Placentae weight (g)[a]	5.3 ± 1.4	4.7 ± 2.7
Litter size[b]	12.0 ± 1.0	12.0 ± 6.2
Fetal weight (g)[b]	1.9 ± 0.2	1.5 ± 0.1*
Pre- implantation loss (%)[b]	0 ± 0	0 ± 1.6
Post- implantation loss (%)[b]	0 ± 8.3	0.5 ± 5.6

*Statistical significance compared to the Control group, $p<0.05$. [a]Values are expressed as mean ± SD. Student T-test.
[b]Values are expressed as median ± interquartile deviation. Mann-Whitney test.
Fonte: Authors

Table 7 - Maternal and fetal parameters in the Control (C) and Topiramate (TPM) groups, with treatment during the organogenesis period (GD7-GD14; Experiment II).

Parameters	Experimental groups (n= 8/group)	
	C	TPM
Body weight of dams (g)		
DG1[a]	274.0 ± 12.3	274.0 ± 14.0
DG7[a]	289.2 ± 14.0	286.6 ± 17.7
DG14[b]	296.0 ± 8.0	307.0 ± 18.0
DG19[a]	371.4 ± 36.1	358.2 ± 22.3
Gravid uterus weight (g)[a]	35.9 ± 7.9	38.2 ± 8.8
Placentae weight (g)[a]	4.7 ± 0.9	5.1 ± 1.3
Litter size[a]	10.2 ± 2.6	11.8 ± 3.1
Fetal weight (g)[b]	1.8 ± 0.1	1.5 ± 0.2*
Pre-implantation loss (%)[b]	0 ± 6.7	0 ± 7.1
Post-implantation loss (%)[a]	2.2 ± 1.1	2.5 ± 1.3

*Statistical significance compared to the Control group, $p<0.05$. [a]Values are expressed as mean ± SD. Student T-test.
[b]Values are expressed as median ± interquartile deviation. Mann-Whitney test.
Fonte: Authors

Discussion

This study evaluated the effects of Topiramate (TPM) on the histology of the ovaries and uterus, and estrous cycle of female rats, as well as its effects on pregnancy outcome of females treated at different stages of pregnancy.

The dose used (100 mg/kg) did not cause toxicity to females, since no clinical signs (piloerection, weakness, tremors, change in behavior) or mortality were observed. According to Richard et al. (2000), doses higher than 100 mg/kg cause potential toxicity, including reduced body weight, decreased water consumption, dehydration, lethargy and stooped posture. In this study, TPM administered for six weeks did not cause significant change in final body weight and ration consumption of females. Similarly, when the drug was administered at different gestational periods, there was no change in maternal body weight. This result corroborate with a previous study (KHOURI, 2005), which showed that the maternal body weight of rats treated with TPM (100 mg/kg) for twelve weeks was similar to the control group. Pavuluri, Janicak e Carbray (2002) reported that the antiepileptic drug also acts as an anorexic agent. However, this effect was not observed in this study.

In the assessment of the vital organs of toxicological interest (kidney, heart and liver), no macropathological change was observed. However, TPM has promoted an increase in the relative weight of the liver and kidney of females. It is reported that TPM promotes renal metabolic acidosis, due to the change in the filtration and reabsorption of bicarbonate (ORUETA et al., 2012). Changes in kidney function may have contributed to an increase in organ weight this study. According to SWANN (2001) most anticonvulsants are metabolized, at least partially, in liver. Therefore, they or their metabolites have the potential for hepatotoxic effects, such as TPM (BJØM et al., 1998). Additional histological analysis would be required to prove a possible toxicity of the drug in the kidney and liver.

In the present study, ovarian weight in the TPM-group has not been affected, when the females received treatment for 6 consecutive weeks, since the folliculogenesis and luteogenesis occurred normally. Then, the treatment with TPM showed no gonadotoxic effect in female Wistar rats. Exposure to the TPM (100 mg/kg) for 4 weeks inexplicably decreased the maternal ovarian weight of Sprague-Dawley rats, while for the longer period (12 weeks) there was an increase in organ weight (KHOURI, 2005). The discrepancy between the results obtained in this study and those reported by Khouri (2005) may be attributed to the differences in animal lineage, drug exposure periods and evaluation of ovarian weight in two different situations, in non-pregnant and pregnant females. The author suggested the need to perform both hormonal and histological analyses, and reported that TPM may act along the hypothalamic-pituitary-ovarian-uterine axis. The mechanisms involved in this result are uncertain. Other drugs, such as valproate (50 or 200 mg/kg; TAUBΦLL et al., 1999) or levetiracetam (50 or 150 mg/kg; SVALHEIM et al., 2008) showed a significant increase in ovarian weight, followed by morphological changes, in non-epileptic female Wistar rats.

In this study, there was no significant effect in the uterine weight of rats treated with TPM for 6 weeks or when the treatment occurred during the various gestational periods (pre-implantation, implantation and organogenesis). Similarly to the reported by Khouri (2005), the decrease in the fetal weights in TPM-group did not significantly influence the uterine weight in comparison to the control group.

Several common antiepileptic drugs (AEDs; e.g. phenobarbital, phenytoin and carbamezepine) may promote abnormalities of baseline endocrine status and lower fertility (HERZOG et al., 2004, 2005). According to Verroti et al. (2011a) it is difficult to determine whether hormonal abnormalities are due to epilepsy-related hypothalamic-pituitary axis dysfunction or to side effects of antiepileptic drugs. However, treatment with certain AEDs, such

as phenobarbital, phenytoin, carbamezepine and valproate may increase the risk of reproductive endocrine disorders in women with epilepsy (VERROTI et al., 2009, 2011a). According to authors, the effects of the new AEDs, including Topiramate, have not been widely studied.

Little information is available about possible associations between AEDs and estrous cycle irregularities. Furthermore, a drug-specific effect of AEDs on ovarian morphology has been studied in detail only for valproate (TAUBΦLL et al., 2008). In this study, TPM *per se* does not affect the estrous cycle of females, indicating that at this dose level and experimental period, it did not promote reproductive endocrine disorders. Thus, ovarian folliculogenesis and luteogenesis occurred normally due to the influence of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), respectively, by the pituitary. Both control and TPM groups, euthanized in estrus phase of the estrous cycle, had the same number of healthy follicles and corpora lutea, although the area of the corpora lutea was significantly higher in the ovaries of the TPM-treated group, probably due to the higher degree of maturation of the luteal structure or presence of generations of corpora lutea from the preceding ovulatory cycles (WESTWOOD, 2008). Regarding atretic follicles, TPM does not induce an increase in number of degenerative follicles in the ovaries, indicating that the drug did not impair follicular development. A previous study (TAUBΦLL et al., 1999) reported that long-term valproate treatment promoted an increase in the number of cystic formations in the ovaries of non-epileptic rats, similarly to polycystic ovaries in women treated with valproate (ISOJÄRVI et al., 1993). Wistar rats treated with levetiracetam, a new AED, present a significantly lower number of cysts and a higher number of corpora lutea, when compared to the control group (SVALHEIM et al., 2008). The action mechanism of TPM, which differs from all other AEDs, could explain the absence of cystic formations in the ovaries of rats in the present study. The results indicate that TPM does not affect the reproductive endocrine function or the kinetics of folliculogenesis in female Wistar rats, and thus may offer an alternative for replacing older AEDs. Moreover, these results demonstrate the absence of adverse effects of the drug on the fertility process of females, as observed in the study performed by Khouri (2005) after the treatment for 4 weeks.

A discrete alteration was observed in uterine tissue of rats treated with TPM, characterized by a decrease in endometrial thickness. This change did not affect the uterine weight. The endometrium presented transitional morphological characteristics between the proliferative and luteal phase of the reproductive cycle. However, these morphological characteristics did not affect the microenvironment required for implantation of the ovum.

Many studies in animals and human have suggested teratogenic effect of TPM, but further investigation is still necessary (PENNEL, 2008; FOUNTAIN, 2009; HERNÁNDEZ-DÍAZ et al., 2012). TPM, gabapentin and leveracetam, newer-generation antiepileptic drugs, do not appear to be major teratogens, but their risks should not be disregarded (MOLGAARD-NIELSEN, HVIID, 2011).

TPM passes freely over the placenta in a one-to-one ratio (OHMAN, 2002). A possible reason for the pathogenesis of teratological changes in fetuses from mothers given TPM during pregnancy is related to histopathological changes in the placenta (MISHRA, SINGH, 2008; OTHMAN et al., 2014). According to the authors, TPM promotes deposition of fibrous material, hemorrhage and increase in the decidual layer thickness, in addition to an increase in the fetal mesenchyme. In the present study, although the placental tissue was not analyzed, the organ weight was similar in the control and TPM groups and there was an absence of gross external morphological defects in fetuses of rats given the drug in different pregnancy periods. However, TPM

promoted a decrease in fetal weight in all periods of exposure (pre-implantation, implantation and organogenesis), suggesting its embryotoxic effect. Previous studies reported that Topiramate reduces fetal weight (KHOURI, 2005; ORNOY et al., 2008). According to Fadel et al. (2012), the reduction of rat fetal weight in TPM-treated groups (50 and 100 mg/kg; day 6-19 of gestation) was accompanied by a parallel decrease in the number of completely ossified vertebral centers, suggesting that TPM produces prenatal growth restriction. Furthermore, maternal weight gain was not significantly affected by TPM treatment, and thus, the reduction in fetal weight was not related to the maternal nutritional status. The results of present study are in agreement with the report by the authors, since the dams did not present reduction in body weight during pregnancy, although the drug decreased the fetal weight.

In this study, the exposure to TPM in different gestational periods did not affect litter size or the pre- and post-implantation losses. The treatment of Sprague-Dawley female rats with Topiramate (100 mg/kg; 12 weeks) during the pre-gestational period decreased the percentage of pregnancies, the number of implantation sites and the number of viable fetuses (KHOURI, 2005). In another study (OTOOM et al., 2004), Sprague-Dawley male rats exposed to TPM diets at a concentration of 100 mg/kg for 60 days presented reduction in sperm motility and density. In addition, the number of female rats impregnated by male rats treated with TPM was decreased, as were the numbers of implantations and viable fetuses. Therefore, these preliminary studies indicate that fetal viability may be reduced when the drug exposure occurs before conception. Its teratogenic effects still merit further attention, as studies already have shown notable effect of the drug in reduction of fetal weight.

In conclusion, the findings indicate that TPM promotes discrete alterations in the uterine tissue of female rats, and causes decrease on the fetus weight after exposure in different gestational periods.

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References

ASTRUP, A.; TOUBRO, S. Topiramate: a new potential pharmacological treatment for obesity. *Obesity Research*, Baton Rouche, v. 12, p. 167S-173S, 2004.

BJØM, K.; GJERSTAD, L.; BENTDAL, Ø.; OSNES, S.; SCHRUMPF, E. Topiramate and fulminate liver failure. *Lancet*, London, v. 352, p. 1119, 1998.

BRAY, G. A.; HOLLANDER, P.; KLEIN, S.; KUSHNER, R.; LEVY, B.; FITCHET, M.; PERRY, B. H. A 6-month randomized, placebo-controlled, dose-ranging trial of topiramate for weight loss in obesity. *Obesity Research*, Baton Rouche, v. 11, p. 722-733, 2003.

CARMO, J. C.; PETER, V. M.; GUERRA, M. O. Cronologia do desenvolvimento embrionário e fetal de ratos conforme a datação do início da prenhez. *Boletim do Centro de Biologia da Reprodução,* Juiz de Fora, v. 23, p. 5-16, 2004.

FADEL, R. A.; SEQUEIRA, R. P.; OBEIDAT, M.; SALEM, A. H. A. Effect of prenatal administration of therapeutic doses of topiramate on ossification of ribs and vertebrae in rat fetuses. *Romanian Journal of Morphology and Embryology*, Bucuresti, v. 53, p. 321-327, 2012.

FOUNTAIN, N. B. A pregnant pause to consider teratogenicity of Topiramate. *Epilepsy Current*, Chicago, v. 9, p. 36-38, 2009.

FRENCH, J. A.; GAZZOLA, D. M. New generation antiepileptic drugs: what do they offer in terms of improved tolerability and safety? *Therapeutic Advances in Drug Safety,* Bethesda, v. 2, p. 141-158, 2011.

HASHISH, H. A. Histopathologic effect of prenatal Topiramate exposure on rat cerebral cortex and hippocampus. *Journal Interdisciplinary of Histopathology,* Ankara, v. 2, p. 61-68, 2014.

HERNÁNDEZ-DÍAZ, S.; SMITH, C. R.; SHEN, A.; MITTENDORF, R.; HAUSER, W. A.; YERBY, M.; HOLMES, L. B. Comparative safety of antiepileptic drugs during pregnancy. *Neurology,* New York, v. 78, p. 1692-1699, 2012.

HERZOG, A. G.; DRISLANE, F. W.; SCHOMER, D. L.; PENNEL, P. B.; BROMFIELD, E. B.; DWORETZKY, B. A.; FARINA, E. L.; FRYE, C. A. Differential effects of antiepileptic drugs on sexual function and hormones in men with epilepsy. *Neurology*, New York, v. 65, p. 1016-1020, 2005.

HERZOG, A. G.; HARDEN, C. L.; LIPORACE, J.; PENNEL, P. B.; SCHOMER, D. L.; SPERLING, M.; FOWLER, K.; NIKOLOV, B.; SCHUMAN, S.; NEWMAN, M. Frequency of catamenial seizure exarcebation in women with localization-related epilepsy. *Annals of Neurology,* Boston, v. 56, p. 431-434, 2004.

HIRSHFIELD, A. N. Development of follicles in the mammalian ovary. *International Review of Cytology*, New York, v. 124, p. 43-101, 1991.

ISOJÄRVI, J. I. T.; LAATIKAINEN, T. J.; PAKARINEN, A. J.; JUNTUNEN, K. T.; MYLLYLÄ, V. V. Polycystic ovaries and hiperandrogenism in women taking valproate for epilepsy. *New England Journal of Medicine*, Boston, v. 329, p. 1383-1388, 1993.

KHOURI, N. A. Reproductive toxic effects of Topamax ingestion in female sprague-dawley rats. *Neuroendocrinology Letters*, Weinheim, v. 26, p. 843-846, 2005.

LIN, K. Topiramato: uma molécula multifacetada. *Revista Neurociências*, São Paulo, v. 19, p. 8-9, 2011.

MISHRA, A.; SINGH, M. Topiramate induced histopathological changes in placenta of rats. *Indian Journal Experimental Biology*, New Delhi, v. 46, p. 715-719, 2008.

MOLGAARD-NIELSEN, D.; HVIID, A. Newer-generation antiepileptic drugs and the risk of major birth defects. *JAMA*, Chicago, v. 305, p. 1996-2002, 2011.

OHMAN, I. Topiramate passess freely over the placenta in a one to one ratio. *Epilepsia*, New York, v. 43, p. 1157-1160, 2002.

ORNOY, A.; ZVI, N.; ARNON, J.; WAJNBERG, R.; SHECHTMAN, S.; DIAV-CITRIN, O. The outcome of pregnancy following Topiramate treatment: A study on 52 pregnancies. *Reproductive Toxicology*, Elmsford, v. 25, p. 388-389, 2008.

ORUETA, L. F.; ESTEBA-FERNÁNDEZ, J.; AICHNER, H. F. J.; CASILLAS-VILLAMOR, A.; RODRÍGUEZ-ÁLVAREZ, S. Acidosis metabólica inducida por topiramato: a propósito de um caso. *Nefrología*, Madrid, v. 32, p. 403-404, 2012.

OSMAN, P. Rate and course of atresia during follicular development in the adult cyclic rat. *Journal of Reproduction and Fertility*, Cambridge, v. 73, p. 261-270, 1985.

OTHMAN, M.; SALEM, A. H.; SEQUEIRA, R.; ABU-HIJLEH, M.; FADEL, R. Effects of the therapeutic doses of Topiramate on the placenta of rats: a histological and morphometric study. *FASEB Journa*l, Bethesda, v. 28, n. 1, 2014.

OTOOM, S.; BATIENEH, H.; HASSAN, Z.; DAOUD, A. Effects of long-term use Topiramate on fertility and growth parameter in adult male rats. *Neuroendocrinology Letters*, Weinheim, v. 25, p. 351-355, 2004.

PAVULURI, M.N.; JANICAK, P.G.; CARBRAY, J. Topiramate plus resperidone for controlling weight gain and symptoms in preschool mania. *Journal of Child and Adolescent Psychopharmacology*, New York, v. 12, p. 271-273, 2002.

PEDERSEN, T.; PETERS, H. Proposal for a classification of oocytes and follicles in the mouse ovary. *Journal of Reproduction and Fertility*, Cambridge, v. 17, p. 208-212, 1968.

PENNEL, P. B. Antiepileptic drugs during pregnancy: what is known and which AEDs seem to be safest? *Epilepsia*, New York, v. 49, p. 1-23, 2008.

PENNEL, P. B. Hormonal aspects of epilepsy. *Neurologic Clinics*, Philadelphia, v. 27, p. 941-965, 2009.

PLOWCHALCK, D. R.; SMITH, B. J.; MATTISON, C. R. Assessment of toxicity to the ovary using follicle quantification and morphometrics. In: HEINDEL, J.; CHAPLIN, R. E. (Ed.). *Methods in toxicology:* female reproductive toxicology. New York: Academic Press Inc., 1993. p. 57-68.

REAGAN-SHAW, S.; NIHA, L. M.; AHMAD, N. Dose translation from animal to human studies revisited. *FASEB Journal*, Bethesda,v. 22, n. 3, p. 659-661, Mar. 2007.

RICHARD, D.; FERLAND, J.; LALONDE, J.; SAMSON, P.; DESHAIES, Y. Influence of Topiramate in the regulation of energy balance. *Nutrition*, Burbank, v. 16, p. 961-966, 2000.

SHAPIRA, N. A.; GOLDSMITH, T. D.; MCELROY, S. L. Treatment of binge-eating disorder with topiramate: a clinical case series. *Journal of Clinical Psychiatry*, Memphis, v. 61, p. 368-372, 2000.

SVALHEIM, S.; TAUBΦLL, E.; SURDOVA, K.; ORMEL, L.; DAHL, E.; ALEKSANDERSEN, M.; MCNEILLY, A.; GJERSTAD, L.; ROPSTAD, E. Long-term levetiracetam treatment affects reproductive endocrine function in female Wistar rats. *Seizure*, London, v. 17, p. 203-209, 2008.

SWANN, A. C. Major system toxicities and side effects of anticonvulsants. *Journal of Clinical Psychiatry*, Memphis, v. 62, p. 16-21, 2001.

TAUBOLL, E.; ROSTE, L. S.; SVALHEIM, S.; GJERSTAD, L. Disorders of reproduction in epilepsy – what can we learn from animal studies? *Seizure*, London, v. 17, p. 120-126, 2008.

TAUBΦLL, E.; ISOJÄRVI, J. I. T.; HARBO, H. F.; PAKARINEN, A. J.; GJERSTAD, L. Long-term valproate treatment induces changes in ovarian morphology and serum sex steroid hormone levels in female Wistar rats. *Seizure*, London, v. 8, p. 490-493, 1999.

TENNIS, P.; CHAN, K. A.; CURKENDALL, S. M.; LI, D. K.; MINES, D.; PETERSEN, C.; ANDREWS, E. B.; CALINGAERT, B.; CHEN, H. Y.; DESHPANDE, G.; EVERAGE, N.; HOLICK, C. N.; MEYER, N. M.; NKOMA, E. T.; QUINN, S.; ROTHMAN, K. J.; ESPOSITO, D. B. Topiramate use during pregnancy and major congenital malformations in multiple populations. *Birth Defects Research Part A*: Clinical and Molecular Teratology, Hoboken, v. 103, p. 268-274, 2015.

VERROTI, A.; D'EGIDIO, C.; MOHN, A.; COPPOLA, G.; PARISI, P.; CHIARELLI, F. Antiepileptic drugs, sex hormones, and PCOS. *Epilepsia*, New York, v. 52, p. 199-211, 2011a.

VERROTI, A.; LA TORRE, R.; TROTTA, D.; MOHN, A.; CHIARELLI, F. Valproate induced insulin resistance and obesity in children. *Hormone Research*, Basel, v. 71, p. 125-131, 2009.

VERROTI, A.; SCAPARROTTA, A.; AGOSTINELLI,S.;DIPILLO,S.;CHIARELLI, F.; GROSSO, S. Topiramate-induced weight loss: a review. *Epilepsy Research*, Amsterdam, v. 95, p. 189-199, 2011b.

WESTWOOD, F. R. The female rat reproductive cycle: a practical histological guide to staging. *Toxicologic Pathology*, Lawrence, v. 36, p. 375-384, 2008.

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