Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v35i1.10946

Testis evaluation of adult Wistar rats after neonatal treatment with fluoxetine

Valdemiro Amaro da Silva Junior*, Waldo Oliveira Monteiro Filho, Catarina Ferreira Pinto, Sandra Maria de Torres and Bruno Mendes Tenorio

*[1]Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n., 52171-900, Dois Irmãos, Recife, Pernambuco, Brazil. *Author for correspondence. E-mail: vajunior@dmfa.ufrpe.br*

ABSTRACT. In current assay the serotoninergic system in newly-born Wistar rats underwent pharmacological modification by fluoxetine, a selective serotonin reuptake inhibitor (SSRI), to investigate its repercussion on testicular parameters in adult animals. Thirty animals were distributed according to treatment: control animals (n = 6), animals treated with 1 mg kg^{-1} (n = 6), 5 mg kg^{-1} (n = 6), 10 mg kg^{-1} (n = 6) and 20 mg kg^{-1} (n = 6) of fluoxetine (IP). When 150 days old, the animals were anesthetized and perfused intra-cardiacally with fixative solution. Testes were routinely processed for inclusion in plastic resin (methacrylate glycol). Further, 4 μm-thick histological sections were stained with toluidine blue/sodium borate 1% and analyzed histometrically. Pharmacological intervention on the serotoninergic system during the postnatal period of the testes development in Wistar rats with fluoxetine chlorohydrate reduced parameters, such as testicular weight, testis liquid weight and seminiferous tubules diameter. However, testicular parameters, such as daily sperm production (DSP), spermatogenesis efficiency (DSP/g/testis) and cell population in stage VII of adult animals, were not influenced by fluoxetine chlorohydrate usage during neonatal period. Results show that administration of fluoxetine during 21 days after birth may induce adverse changes in the spermatogenesis of adult rats.

Keywords: spermatogenesis, Sertoli cell, sperm production, fluoxetine chlorohydrate.

Avaliação testicular de ratos Wistar adultos após tratamento neonatal com fluoxetina

RESUMO. No presente trabalho, o sistema serotoninérgico de ratos Wistar recém-nascidos foi farmacologicamente modificado por um inibidor seletivo de recaptação da serotonina, fluoxetina, com o objetivo de observar sua repercussão nos parâmetros testiculares em ratos adultos. Trinta animais foram distribuídos de acordo com o tratamento: controle (n = 6), tratado com 1 mg kg^{-1} (n = 6), 5 mg kg^{-1} (n = 6), 10 mg kg^{-1} (n = 6) e 20 mg kg^{-1} (n = 6) de fluoxetina. Aos 150 dias de vida, os animais foram anestesiados e perfundidos intracardiacamente com solução fixadora. Os testículos foram removidos e processados para inclusão em resina plástica (glicol metacrilato). Cortes histológicos com 4 μm de espessura foram corados por azul de toluidina/borato de sódio 1% e analisados histometricamente. O tratamento com fluoxetina reduziu nos parâmetros de peso testicular líquido e bruto, bem como no diâmetro dos túbulos seminíferos. Entretanto, os parâmetros testiculares de produção espermática diária (PED), eficiência espermática (PED/g/testículo) e população de células germinativas no estágio VII não estavam alteradas pelo tratamento com fluoxetina. Em conclusão, a administração de fluoxetina durante 21 dias após o nascimento pode induzir efeitos adversos na espermatogênese de ratos adultos.

Palavras-chave: espermatogênese, células de Sertoli, produção espermática, cloridrato de fluoxetina.

Introduction

During the last few years there has been an increase in interest on the collateral effects caused by medicines that act on brain neurotransmitters, such as serotonin, dopamine and noradrenalin. The evaluation on the profile of these collateral effects induced by antidepressants and neuroleptic medicines is particularly interesting, especially if the important information such evaluation may provide on the therapeutic activity of these pharmacological agents is taken into account (RÉNYI, 1986).

Selective serotonin reuptake inhibitors (SSRIs) are the most common antidepressants prescribed for depression (BALDESSARINI, 1996) and the main collateral effect observed in the anti-depression therapy with SSRIs is associated with sexual dysfunctions (WALDINGER; OLIVER, 1998).

According to Matuszcyk et al. (1998), rats submitted to subchronic doses of fluoxetine presented a significant reduction in sexual motivation and an increase in ejaculatory latency. Cantor et al. (1999) observed that rats treated with

subchronic doses of fluoxetine had decreased ejaculatory responses, although this collateral effect was attenuated by oxytocin. In human beings, orgasm and erection are affected by SSRI antidepressants after 3 months of administration (ROSEN et al., 1999).

In general, monoaminoxidase inhibitors (MAOi), tricycles or SSRIs may induce suppression on reproductive axis (LABBATE et al., 1998; PERRY; FULLER, 1997; TORRES et al., 1998). This suppression may be caused by the simultaneous activation of hypothalamus-pituitary-adrenal (HPA) and sympathetic-adrenomedular axis. The most important factors involved are CRH (Corticotrophin released hormone), endogenous opioid peptides (EOP), catecholamines and glucocorticoids (CALOGERO et al., 1998; CHROUSOS, 1998).

Pharmacological evidences indicate that increase of the 5-HT (5-hidroxitryptamine) levels in the central nervous system affects secretion of FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) by the inhibition of GnRH (Gonadotropin Releasing Hormone), with effects over spermatogenesis and steroidogenesis in adult rats (DAS et al., 1985).

Despite knowledge on the activity of serotonin and noradrenalin on hypothalamus-pituitary-gonad axis or its directly action on testicular functions of sexually mature rats (HEDGER et al., 1995) and on the collateral effects produced by antidepressants (BALDESSARINI, 1996), to date, studies have not been undertaken on the pharmacological manipulation in serotoninergic system during the critical period of testicular development in neonate rats and its reflexes in the spermatogenic process of sexually mature rats.

According to Lesage et al. (1996) , neonate male rats have an activation of HPA and HPG (hypothalamus-pituitary-gonad) axis which may be temporarily related to an intra-hypothalamic increase of the serotonin, noradrenalin and NPY (Neuropeptide Y). The HPG axis function in neonate rats is determinant to the proliferation of Sertoli cells and the establishment of sperm production in adult rats (ROCHA et al., 1999).

Adequate levels of FSH in neonate rats are crucial for the establishment of Sertoli cell population, which is directly related to testis size and spermatic production (FRANÇA et al., 2000). Effects caused by pharmacological intervention on serotoninergic system in neonatal period on the testis development should be better investigated. Current study evaluates the effects caused by neonatal administration of fluoxetine chlorohydrate during the critical period of testis development and its consequences over the testis functionality in sexually mature rats.

Material and methods

Animals, pharmacological manipulation and experimental design

Fifteen adult female rats and five adult male rats obtained from vivarium of the Department of Animal Morphology and Physiology of UFRPE were divided into 5 groups each with 3 females per 1 male adult rat. They were mated overnight and vaginal smear was made to find sperm in vaginal tract. Positive females were separated 3 per cage and weighted weekly until the birth of pups. After birth, male pups were chosen randomly and grouped six per cage with their respectively mothers, until the twenty-first day of birth.

Thirty neonate Wistar rats (*Rattus norvegicus*, var. *albinus*) were arranged in the following groups: I) rats treated with 1 mg Kg^{-1} of fluoxetine chlorohydrate (n = 6); II) rats treated with 5 mg kg^{-1} of fluoxetine chlorohydrate (n = 6); III) rats treated with 10 mg kg^{-1} of fluoxetine chlorohydrate (n = 6); IV) rats treated with 20 mg kg^{-1} of fluoxetine chlorohydrate (n = 6); V) control group (n = 6) treated with sterile de-ionized water.

The male pups from treated groups were injected ip with fluoxetine chlorohydrate (FRANK et al., 2000; GANDARIAS et al., 1999; MATUSZCYK et al., 1998) from the 1st day after birth up to the 21st day of age. After weaning, each group had free access to pelletized food and water until the end of the experiment, at the age of 150 days. They were kept in a 12h light/dark cycle, with controlled humidity (50%) and temperature (22ºC) in the vivarium of the Department of Animal Morphology and Physiology of UFRPE.

The experimental protocol was approved by the Committee on Animal Research and Ethics of the Federal Rural University of Pernambuco (TRADUZIDO) (CEEUA/DMFA 014/2002).

Tissue fixation and histological processing

When the animals were 150 days old, they were injected intraperitoneally with heparin at a dose of 125UI 100 g^{-1} of BW. Adult rats were anesthetized with thiopental (50 mg kg^{-1}) and perfused through the left ventricle with 0.9% NaCl solution during 5 to 10 minutes for clearance of blood vessels. The animals were then perfused with glutaraldehyde 4% in a phosphate buffer solution 0.01 M, pH 7.4. After dissection of testicles and epididymis, both organs

were weighted. The former was sliced into fragments 2 mm thick and re-fixed in the same fixative solution. Testicular tissue was processed in plastic resin (glycol methacrylate -Leica). Further, 4 μm thick histological sections were stained with toluidine blue/sodium borate (1%) and analyzed. Histometrical analysis of testis, determination of Sertoli cell number and calculation of spermatic daily production, based on quantitative histology of testis were undertaken, according to Silva Junior et al. (2006) and Moraes et al. (2009).

Volume densities (%) of testis components

Volume densities of several testicular tissue components were determined using a 441-intersection grid placed in the ocular of a light microscope. Fifteen fields chosen randomly (6615 points) were scored for each animal at 400X magnification. Points were classified as one of the following: seminiferous tubule (tunica propria, germinative epithelium and lumen), Leydig cell, connective tissue, blood and lymphatic vessels. The volume of each testicular component was determined as the product of volume density and testis volume. The volume of each testicular component (mL) was calculated from previous knowledge of their percentile occupied in testis and the testis net weight. According França and Russell (1998) the testis net weight in rats was determined by the reduction of 6.5% from its gross weight. Reduction is related to percentile of albuginea and mediastinum weight in rat testis (Testis Net Weight = Testis gross weight - 6.5%).

Tubular diameter, seminiferous epithelium height and seminiferous tubules total length

The average diameter of 30 cross-sections of round seminiferous tubules per animal was obtained with a linear reticule micrometer (U-OCMSQ10/10, Olympus) coupled to an ocular with 10X magnification and an objective with 10X magnification (100X final magnification). In the same cross-sections used to measure the tubular diameter, the height of the seminiferous epithelium was measured from the membrane base to the tubular lumen. The height of the epithelium in each tubule represented the average of the diametrically opposite measurements. The total length (in meters) of seminiferous tubules (LST) per testis was estimated by the tubules seminiferous volume (TSV) in the testis and the average area of tubules obtained from each animal (πR^2; R = tubular diameter/2), according to the formula: LST = TSV/πR2 (ATTAL; COUROT, 1963; DORST; SAJONSKI, 1974).

Testis Morphometry (cell counts)

The germ cell nuclei (spermatocyte I in preleptotene/leptotene (SPT I Pl/L); spermatocyte I in pachytene (SPT I P); round spermatids (SPD Ar) and Sertoli cell nucleolus at stage VII (RUSSELL et al., 1990) were counted in 10 round seminiferous tubule cross-sections, chosen at random for each animal. These counts were corrected for section thickness and for nucleus or nucleolus diameter, according to Amann and Almquist (1962):

$$Corrected\ number = counted\ score \times \frac{Cut\ thickness}{Cut\ thickness + \sqrt{\left(\frac{AD^2}{2}\right) - \left(\frac{AD^2}{4}\right)}}$$

Estimates on Sertoli cell number per testicle were performed based on the Sertoli cell nucleolus corrected number per seminiferous tubule transversal section in stage VII and the total length of seminiferous tubule per testicle, following the formula by Hochereau-de Reviers and Lincoln (1978):

$$SC\ Number / testicle = \frac{Sem.\ Tub.\ Tot.\ Len. \times Cor.\ Numb.\ SC\ Nucl. / Transv.\ Sec.}{Cut\ Thickness}$$

where,

SC Number / testicle = Sertoli cell number per testicle; Sem. Tub. Tot. Len. = Seminiferous Tubules Total Length (μm); Cor. Numb. SC Nucl. / Transv. Sec. = Corrected Number of Sertoli Cell Nucleolus per Transversal Section; Cut thickness (μm). Daily spermatic production per testicle and per gram of testicle was obtained, following Silva Junior et al. (2006):

$$DSP = \frac{SC\ Total\ No / testide \times RS\ No \times Rel.\ Freq.Stage\ VII}{Stage\ VIIDuration}$$

where,

DSP = Daily Spermatic Production; SC Total No / testicle = Sertoli Cell Total Number per testicle; RS No = Round Spermatids Number in stage VII; Rel. Freq. Stage VII = Relative Frequency of Stage VII (%); Stage duration (days). The daily sperm production per gram of testis was obtained by the ratio between DSP and testicular net weight.

Statistical analysis

Shapiro-Wilk test checked the trend to normality of the obtained data. Subsequently, depending on the normal trend of the results, parametric or nonparametric test was employed. Analysis of variance (ANOVA) with Tukey-Kramer post-hoc test was undertaken for normal data. If the data failed to follow

a normal trend, the nonparametric test of Kruskal-Wallis with Dunn post-hoc test was employed. Data were expressed as mean and (±) standard deviation. All statistical analysis was outlined for $p < 0.05$.

Results and discussion

In current experiment no significant difference in final corporal weight was reported between control group and that of the groups treated with different doses of fluoxetine chlorohydrate. Silva Junior et al. (2008) observed slight decrease in the final corporal weight at the end of the same treatment. Previous studies with citalopram and fluoxetine, through subcutaneous administration, for the same treatment period decreased body weight gain (DEIRÓ et al., 2004; MENDES DA SILVA et al., 2002). This result may be related to the inhibitory action of serotonin on food ingestion (SIMANSKY, 1995) although, according to Morrison et al. (2005), a reduction on intestinal villus height caused by the administration of selective serotonin reuptake inhibitors would cause a decrease in nutrient absorption. Even though the phenomenon of weight gain decrease had already been described for postnatal development in rats, the interruption in treatment seemed to have influenced the recovery of body weight, since no difference between the animals' weight was found in current study.

Fluoxetine chlorohydrate administrated in male pups during 21 postnatal days caused a significant reduction of 8, 10, 14 and 13% on the testicle weight of groups treated with increasing doses, 1, 5, 10 and 20 mg kg^{-1}, respectively, when compared to control group aged 150 days. A reduction of testicular weight among animals treated with 10 mg kg^{-1} was observed when compared to the group treated with lowest fluoxetine dose (Table 1). According to França and Russell (1998), testicular weight is a morphometrical parameter directly and positively related to seminiferous tubules total length, Sertoli cell population and spermatic production. In rats, fetal and neonatal period are very important for establishing the testis's final size and spermatic production in sexually mature animals (ORTH, 1993). The Sertoli cell population and other parameters such as testicular weight, seminiferous tubules length and sperm production in adult animal during both periods are similarly established (SILVA JUNIOR et al., 2006, 2008).

No statistical difference among groups was established with regard to weight of epididymis and gonadosomatic index (SGI) (Table 1).

Some significant decrease in testis net weight (mg) and in testicular seminiferous tubules volume among animals treated with 5, 10 and 20 mg was reported when testicular volumetric parameters were evaluated and compared to those of control group. There was also a similar decrease in seminiferous epithelium volume in animals treated with 1, 5 and 10 mg when compared to that in control group. Further, other volumetric parameters of testicular parenchyma did not show any alterations (Table 2). The seminiferous tubules in rats constitute approximately 89% of testicular parenchyma.

Table 1. Body and testicular weight and GSI (Gonadosomatic index) of control Wistar rats and rats treated with different fluoxetine doses at 150 days old.

	Control (n = 6)	Fluoxetine (1 mg kg^{-1}) (n = 6)	Fluoxetine (5 mg kg^{-1}) (n = 6)	Fluoxetine (10 mg kg^{-1}) (n = 6)	Fluoxetine (20 mg kg^{-1}) (n = 6)
Body Weight (g)	397.7 ± 70.7	387.33 ± 31.58	388.7 ± 13.3	351.2 ± 28.0	388.5 ± 39.7
Testicular Weight (g)	1.78 ± 0.11a	1.63 ± 0.06b	1.60 ± 0.08bc	1.53 ± 0.07c	1.55 ± 0.11bc
Epididymis (g)	0.63 ± 0.05	0.62 ± 0.03	0.63 ± 0.03	0.60 ± 0.02	0.63 ± 0.05
SGI (%)*	0.46 ± 0.09	0.42 ± 0.03	0.41 ± 0.01	0.44 ± 0.05	0.40 ± 0.03

Different letters in same line indicate significant statistical differences. *SGI= (Gross testis/body weight) x 100.

Table 2. Volumetric parameters of testicular parenchyma (mL) components in control Wistar rats control and rats treated with different doses of Fluoxetine, at 150 days old.

	Control (n = 6)	Fluoxetine (1 mg kg^{-1}) (n = 6)	Fluoxetine (5 mg kg^{-1}) (n = 6)	Fluoxetine (10 mg kg^{-1}) (n = 6)	Fluoxetine (20 mg kg^{-1}) (n = 6)
Net testicular weight	1.67 ± 0.1a	1.52 ± 0.06b	1.49 ± 0.07b	1.42 ± 0.1b	1.44 ± 0.1b
Sem. Tubules	1.54 ± 0.12a	1.41 ± 0.06ab	1.35 ± 0.09b	1.31 ± 0.06b	1.34 ± 0.12b
Sem. Epithelium	1.39 ± 0.11a	1.26 ± 0.06b	1.24 ± 0.09b	1.21 ± 0.07b	1.23 ± 0.10ab
Lumen	0.11 ± 0.03	0.12 ± 0.04	0.08 ± 0.02	0.08 ± 0.05	0.08 ± 0.03
Tunica Propria	0.039 ± 0.006	0.033 ± 0.009	0.033 ± 0.004	0.029 ± 0.006	0.028 ± 0.007
Leydig	0.024 ± 0.005	0.027 ± 0.006	0.034 ± 0.007	0.025 ± 0.007	0.032 ± 0.006
Conjun. Cells	0.003 ± 0.003	0.003 ± 0.001	0.003 ± 0.001	0.004 ± 0.003	0.002 ± 0.002
Blood vases	0.034 ± 0.021	0.045 ± 0.022	0.034 ± 0.012	0.033 ± 0.011	0.025 ± 0.008
Lymphatic Space	0.066 ± 0.033	0.036 ± 0.020	0.072 ± 0.022	0.049 ± 0.016	0.046 ± 0.016

Different letters in same line indicate significant statistical differences.

These data are correlated to testis net weight, seminiferous tubules volume, seminiferous epithelium, total length of seminiferous tubules and spermatic production (FRANÇA et al., 2005). In current experiment, the seminiferous tubules volume was altered in animals treated with doses starting from 5 mg kg^{-1} of fluoxetine, with an approximate 13.42% average reduction of this parameter. These results corroborate the findings of Silva Junior et al. (2008) who manipulated the serotoninergic system with fluoxetine in neonatal rats during the first 21 postnatal days and analyzed the testicles morphometrically on the 22nd postnatal day. A reduction in seminiferous tubule, directly influenced by the Sertoli cell population, was reported.

Germ cells counting were made at stage VII of the seminiferous epithelium cycle. Spermatogonia A, spermatocyte I in pre-leptotene, spermatocyte I in pachytene, round spermatids and elongated spermatids bordering the lumen and attached to the apical pole of Sertoli cell may be found at this stage. Cell population per cross-section of seminiferous tubule at stage VII of seminiferous epithelium cycle may be observed in Table 3. According to morphometrical evaluation, no statistical difference was found between experimental groups and control.

Table 4 shows values related to biometric parameters from testicular parenchyma, Sertoli cell population and spermatic production per testicle and per gram of testicle in Wistar rats from control group and groups treated with different doses of fluoxetine. Results in Table 4 show that a statistically significant reduction occurred on tubular diameter in the group treated with 20 mg kg^{-1} of fluoxetine when compared to that of the remaining groups.

Tubular diameter and seminiferous epithelium height reflects different degrees of epithelium activity. These parameters are highly important to quantitative evaluation of spermatogenesis when there is a positive correlation between tubular diameter and testicle spermatogenic activity (MORAES et al., 2009). França and Russell (1998) said that tubular diameter cannot be altered significantly after sexual maturity have been established and it is constant throughout the stages of seminiferous epithelium cycle in most species, even though expressive interspecies or racial variations occur. Silva Junior et al. (2008) did not observe changes in tubular diameter in testis of 22-day-old rats after treatment with fluoxetine. However, the 60-day long-term ingestion of fluoxetine (200 mg kg^{-1}) greatly decreased spermatogenesis in seminiferous tubules. In fact, a germ cell reduction in the treatment group was observed (BATAINEH; DARADKA, 2007). Probably the 21-day short-term treatment with fluoxetine was not enough to produce changes in tubular diameter in 150-day-old adult rats.

Table 3. Cell population per seminiferous tubules transversal section in stage VII of seminiferous epithelium cycle in 150 days old Wistar rats treated with different doses of Fluoxetine.

	Control (n=6)	Fluoxetine (1 mg kg^{-1}) (n=6)	Fluoxetine (5 mg kg^{-1}) (n=6)	Fluoxetine (10 mg kg^{-1}) (n=6)	Fluoxetine (20 mg kg^{-1}) (n=6)
Spermatogone A	0.92 ± 0.35a	0.77 ± 0.2a	0.7 ± 0.1a	0.8 ± 0.1a	0.8 ± 0.2a
Sertoli Cell Nucleolus	9.4 ± 1.7a	8.7 ± 1.8a	10.3 ± 1.4a	8.8 ± 0.6a	8.1 ± 0.9a
Spermatocyte I Pre-leptotene	25.2 ± 4.9a	26.0 ± 3.2a	24.6 ± 5.6a	29.3 ± 3.2a	27.0 ± 1.7a
Spermatocyte I Pachytene	31.8 ± 5.1a	27.3 ± 6.9a	28.5 ± 3.6a	28.8 ± 2.2a	28.5 ± 2.4a
Round Spermatids	83.5 ± 15.1a	74.2 ± 7.1a	78.2 ± 10.1a	80.8 ± 7.8a	72.9 ± 9.8a

Different letters in same line indicate significant statistical differences.

Table 4. Biometric and morphometric data in 150-day-old Control and Fluoxetine-treated rats (n = 6 rats per group; means ± SEM).

	Control (n = 6)	Fluoxetine (1 mg kg^{-1}) (n = 6)	Fluoxetine (5 mg kg^{-1}) (n = 6)	Fluoxetine (10 mg kg^{-1}) (n = 6)	Fluoxetine (20 mg kg^{-1}) (n = 6)
Testis net weight(g)	1.67 ± 0.1a	1.52 ± 0.06b	1.49 ± 0.07b	1.42 ± 0.1b	1.44 ± 0.1b
Tubular Diameter (μm)	384.6 ± 27.1a	365.6 ± 15.1a	388.6 ± 11.3a	366.0 ± 46.0a	318.9 ± 26.9b
Epithelium Height (μm)	117.9 ± 7.4abc	114.2 ± 5.0abc	121.7 ± 12.1b	102.0 ± 14.3c	107.6 ± 8.7abc
Seminiferous Tubule T. Length (m)	13.4 ± 1.9ab	13.5 ± 1.1ab	11.4 ± 1.3a	12.9 ± 3.0a	17.0 ± 2.6b
Sertoli/ cross section	9.4 ± 1.7	8.7 ± 1.8	10.3 ± 1.4	8.8 ± 0.6	8.1 ± 0.9
Round SPD/ cross section	83.51 ± 15.6	74.2 ± 7.1	78.2 ± 10.1	80.8 ± 7.8	72.9 ± 9.8
SCI	8.9 ± 0.06	8.4 ± 1.2	7.7 ± 1.3	9.2 ± 1.2	9.1 ± 1.6
Sertoli/Testicle (x10^7)	3.12 ± 0.56	2.95 ± 0.71	2.97 ± 6.9	2.86 ± 0.75	3.48 ± 0.79
DSP/Testicle (x10^6)	20.7 ± 3.8	18.8 ± 2.3	16.8 ± 3.2	19.3 ± 3.3	23.4 ± 6.2
DSP/g/Testicle (x10^6)	12.4 ± 1.8ab	12.3 ± 1.6ab	11.2 ± 1.8b	13.6 ± 2.6ab	16.1 ± 3.7ac

Different letters in same line indicate significant statistical differences. SCI = Sertoli Cell Index; DSP = Daily Sperm Production.

On the other hand, seminiferous epithelium height was significantly different between groups treated with 5 and 10 mg kg^{-1} of fluoxetine. Animals treated with 10 mg kg^{-1} of fluoxetine showed 16% of reduction on seminiferous epithelium height when compared to those treated with 5 mg kg^{-1} of fluoxetine. Rats treated with the highest dose of fluoxetine tended to present a reduction around 12% when compared to that in group 5 mg kg^{-1} of fluoxetine, although no significant difference between treated groups and control group was reported.

Seminiferous epithelium height in most domestic species shows few variations related to the diverse composition of cellular associations or possible alterations on Sertoli cell volume seen at different stages of seminiferous epithelium cycle (FRANÇA; RUSSELL, 1998). Furthermore, various etiologic factors may be involved in the degeneration of developing testicular. In fact, advanced age, nutritional deficiency, heat shock, hormones, neoplasias, irradiation, trauma and others interrupt the spermatogenic process, initially characterized by germinative cells desquamation in tubular lumen and by a decrease in seminiferous epithelium height, necrosis and apoptosis of germinative epithelium cells and hyalinization of seminiferous tubules (ORTEGA-PACHECO et al., 2006). However, no alteration that justified testicular degeneration was reported. Neonatal treatment did not alter in adult rats the cell population per transversal section in stage VII of seminiferous epithelium cycle. According to Bataineh and Daradka, (2007), the long-term use of fluoxetine in high doses could cause germ cell degeneration and decrease of seminiferous epithelium height. The fluoxetine usage in the neonate rats during 21 days did not produce alterations in epithelium height in 150-day-old adult rats, probably due to short-term and dose of fluoxetine used in current assay.

In the case of total seminiferous tubules length, a significant reduction occurred in animals treated with 5 and 10 mg kg^{-1} when compared to group treated with 20 mg. Daily sperm production per gram of testicle (DSP g^{-1} of testicle), which estimates efficiency of spermatogenic process, was significantly reduced in animals treated with 5 mg when compared to those treated with 20 mg (Table 4).

Other parameters like Sertoli cells and number of round spermatids per cross-section of seminiferous tubule in stage VII of S.E.C. were not affected, owing to neonatal treatment with fluoxetine in crescent dosages. Sertoli cells support capacity or Sertoli cells index (SCI) were not affected too. Total Sertoli cell population per testicle was not influenced by the administration of different doses of fluoxetine in neonatal period as well as by daily spermatic production per testicle. On the other hand, a rise in spermatogenic process efficiency (daily sperm production/gram of testicle) could be observed in animals that received the highest dose when they were compared to animals treated with 5 mg kg^{-1} of fluoxetine.

An approximately 15.5% tubular diameter reduction observed in animals treated with the highest dose of fluoxetine may justify many of the findings such as the increase in tubular length and the maintenance of parameters like daily spermatic production per testicle, per gram of testicle and Sertoli cell population. On the other hand, in current experiment testicular weight, seminiferous tubules and seminiferous epithelium volume were reduced in fluoxetine-treated animals. The above findings may also demonstrate that reduction on Sertoli cell population, total seminiferous tubules length, daily spermatic production per testicle and spermatogenesis efficacy should have occurred too. According to Silva Junior et al. (2008), the manipulation of serotoninergic system in rats during testicular critical development period reduced Sertoli cell population and FSH levels in pre-pubescent animals. According to the literature, some reduction in morphometric parameters directly related to this cell population on adult subjects should be expected (FRANÇA et al., 2000; FRANÇA et al., 2005; SILVA JUNIOR et al., 2006). High doses of fluoxetine and their long-term usage in adult rats were closely related with decrease testosterone levels, FSH levels and testis degeneration (BATAINEH; DARADKA, 2007). Therefore, the fluoxetine chlorohydrate could change the pituitary-hypothalamic-gonadic axes regardless of age, although the consequences seem to be different according to time of use and dose per kilogram of weight.

Notwithstanding what has been observed in current experiment, a conflict exists to the information commonly found in the literature. After suspending the selective serotonin reuptake inhibitor (SSRI) and after fluoxetine and norfluoxetine was eliminated in neonates after 5 to 15 hours, respectively (CACCIA et al., 1990), the testis may have developed adaptation strategies, which promoted a recovery in morphometrical and cellular species patterns.

FSH (Follicle Stimulating Hormone) is an important to Sertoli cell proliferation (ALMIRÓN; CHEMES, 1988; ORTH, 1993) and necessary for the establishment of the final size of testis and spermatic production (ORTH, 1993). According to Silva Junior et al. (2008), the reduction on FSH

levels and Sertoli cell population at the 21st postnatal day did not reduce sperm production and spermatogenesis efficiency in adult animals. This statement is due to the fact both experiments followed the same treatment protocol although analysis was made at different periods of testicular development. A recovery in testicular parameters may have occurred in current assay, which was probably related to the rise in FSH levels at the end of treatment with SSRI in animals during the 21 postnatal days and to an extended sensibility period of Sertoli cell to this hormone.

Conclusion

Pharmacological intervention on serotoninergic system during neonatal testis critical development period in Wistar rats with fluoxetine chlorohydrate interfered in parameters such as testicular weight, testis net weight and seminiferous tubules diameter. However, productive morphometric parameters as testicular daily spermatic production, spermatogenesis efficiency per gram of testis and cell population in stage VII of adult animals were not influenced by fluoxetine chlorohydrate usage during neonatal period. Results show that fluoxetine administration during 21 days after birth may induce some adverse changes in adult rat testis.

Acknowledgements

CNPq financial support; Farmácia Roval de Manipulação; UFRPE (*Universidade Federal Rural de Pernambuco*).

References

ALMIRÓN, I.; CHEMES, H. Spermatogenic onset. II. FSH modulates mitotic activity of germ and Sertoli cells in immature rats. **International Journal of Andrology**, v. 11, n. 3, p. 235-246, 1988.

AMANN, R. P.; ALMQUIST, J. O. Reproductive capacity of dairy bulls. VIII. Direct and indirect measurement of testicular sperm production. **Journal of Dairy Science**, v. 45, n. 6, p. 774-781, 1962.

ATTAL, J.; COUROT, M. Developpement testiculaire et etablissement de la spermatogeneses chez le taureau. **Annales de Biologie Animale, Biochimie, Biophysique**, v. 3, n. 3, p. 219-241, 1963.

BALDESSARINI, R. J. Fármacos e o tratamento dos distúrbios psiquiátricos (depressão e mania). In: GOODMAN, L. S. (Ed.). **As bases farmacológicas da terapêutica**. 9. ed. México: Mc Graw-Hill Companies, 1996. Cap. 19, p. 314-334.

BATAINEH, H. N.; DARADKA, T. Effects of long-term use of fluoxetine on fertility parameters in adult male rats. **Neuroendocrinology letters**, v. 28, n. 3, p. 321-325, 2007.

CACCIA, S.; CAPPI, M.; FRACASSO, C.; GARATTINI, S. Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. **Psychopharmacology**, v. 100, n. 4, p. 509-14, 1990.

CALOGERO, A. E.; BAGDY, G.; D'AGATA, R. Mechanisms of Stress on Reproduction: Evidence for a Complex Intra-Hypothalamic Circuit. **Annals of the New York Academy of Science**, v. 851, n. 1, p. 364-370, 1998.

CANTOR, J. M.; BINIK, Y. M.; PFAUS, J. G. Chronic fluoxetine inhibits sexual behavior in the male rat: reversal with oxytocin. **Psychopharmacology**, v. 144, n. 4, p. 355-362, 1999.

CHROUSOS, G. P. Stressors, Stress, and Neuroendocrine Integration of the Adaptive Response: The 1997 Hans Selye Memorial Lecture. **Annals of the New York Academy of Science**, v. 851, n. 1, p. 311-335, 1998.

DAS, T. K.; MAZUNDER, R.; BISWAS, N. M. Effect of intraventricular injection of 5,6-dihydroxytryptamine on spermatogenesis and plasma testosterona levels in rat. **Journal of Endocrinology**, v. 106, n. 3, p. 395-400, 1985.

DEIRÓ, T. C. B. J.; MANHÃES, CASTRO, R.; CABRAL FILHO, J. E.; SOUZA, S. L.; FREITAS, S.; FERREIRA, L. M. P.; GUEDES, R. C. A.; CÂMARA, C. R. V.; BARROS, K. M. F. T. Neonatal administration of citalopram delays somatic maturation in rats. **Brazilian Journal of Medical and Biological Research**, v. 37, n. 10, p. 1503-1509, 2004.

DORST, V. J.; SAJONSKI, H. Morphometrische untersuchunhen am tubulussystem des schweinehodens während der postnatalen entwicklug. **Monatsh Veteterinay Medical**, v. 29, p. 650-652, 1974.

FRANÇA, L. R.; RUSSELL, L. D. The testis of domestic mammals. In: MARTINEZ-GARCIA, F.; REGADERA, J. (Ed.). **Male reproduction**: a multidisciplinary overview. Madrid: Churchill Comunictions Europe España, 1998. Cap. 16, p. 198-219.

FRANÇA, L. R.; AVELAR, G. F.; ALMEIDA, F. F. L. Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. **Theriogenology**, v. 63, n. 2, p. 300-318, 2005.

FRANÇA, R. L.; SILVA JR., V. A.; CHIARINI-GARCIA, H.; GARCIA, S. K.; DEBELJUK, L. Cell proliferation and hormonal changes during postnatal development of the testis in the pig. **Biology of Reproduction**, v. 63, n. 6, p. 1629-1636, 2000.

FRANK, J. L. W.; HENDRICKS, S. E.; OLSON, C. H. Multiple ejaculations and chronic fluoxetine effects on male rat copulatory behavior. **Pharmacology Biochemistry and Behavior**, v. 66, n. 2, p. 337-342, 2000.

GANDARIAS, J. M.; ECHEVARRIA, E.; ACEBES, I.; ABECIA, L. C.; CASIS, O.; CASIS, L. Effects of fluoxetine administration on μ-opiod receptor immunostaining in the rat forebrain. **Brain Research**, v. 817, n. 1-2, p. 236-240, 1999.

HEDGER, M. P.; KHATAB, S.; GONZALES, G.; KRETSER, D. M. Acute and shot-term action of

serotonin administration on the pituitary-testicular axis in the adult rat. **Reproduction fertility development**, v. 7, n. 5, p. 1101-1109, 1995.

HOCHEREAU-DE REVIERS, M. T.; COUROT, M. Sertoli cell and development of seminiferous epithelium. **Annales de Biologie Animale, Biochimie, Biophysique**, v. 18, n. 2B, p. 573-583, 1978.

LABBATE, L. A.; GRIMES, J. B.; ARANA, G. W. Serotonin reuptake antidepressant effects on sexual function in patients with anxiety disorders. **Biology Psychiatry**, v. 43, n. 12, p. 904-907, 1998.

LESAGE, J.; BERNET, F.; MONTEL, V.; DUPOUY, J. P. Effects of prenatal morphine on hypothalamic metabolism of neurotransmitters and gonadal and adrenal activities, during the early postnatal period in the rat. **Neurochemical Research**, v. 21, n. 6, p. 723-732, 1996.

MATUSZCYK, J. V.; LARSSON, K.; ERIKSSON, E. The selective serotonin reuptake inhibitor fluoxetine reduces sexual motivation in male rats. **Pharmacology Biochemistry and Behavior**, v. 60, n. 2, p. 527-532, 1998.

MENDES DA SILVA, C.; SOUZA, S. L.; BARRETO MEDEIROS, J. M.; FREITAS-SILVA, S. R.; ANTUNES, D. E. C.; CUNHA, A. D. U.; RIBAS, V. R.; FRANÇA, M. F. S.; NOGUEIRA, M. I.; MANHÃES DE CASTRO, R. Neonatal treatment with fluoxetine reduces depressive behavior induced by forced swim in adult rats. **Arquivos de Neuropsquiatria**, v. 60, n. 4, p. 928-931, 2002.

MORAES, T. A. P.; JASSET, P. F.; TORRES, S. M.; MORAES, A. V.; SILVA JÚNIOR, V. A.; GUERRA, M. M. P. Efeito do uso de pentoxifilina no período neonatal sobre a produção espermática em ratos Wistar adultos. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 61, n. 1, p. 80-87, 2009.

MORRISON, J. L.; RIGGS K. W.; RURAK D. W. Fluoxetine during pregnancy: impact on fetal development. **Reproduction Fertility and Development**, v. 17, n. 6, p. 641-650, 2005.

ORTEGA-PACHECO, A.; RODRÍGUEZ-BUENFIL, J. C.; SEGURA-CORREA, J. C.; BOLIO-GONZALEZ, M. E.; JIMÉNEZ-COELLO, M.; LINDE FORSBERG, C. Pathological conditions of the reproductive organs of male stray dogs in the tropics: prevalence, risk factors, morphological findings and testosterone concentrations. **Reproduction in Domestic Animals**, v. 41, n. 5, p. 429-437, 2006.

ORTH, J. M. Cell biology of testicular development in fetus and neonate. In: DESJARDINS, C.; EWING, L. L. (Ed.). **Cell and molecular biology of the testis**. 1st ed. New York: Oxford University Press, 1993. p. 3-42.

PERRY, K. W.; FULLER, R. W. Fluoxetine increases norepinephrine release in rat hypothalamus as measured by tissue levels of MHPG-SO_4 and microdialysis in conscious rats. **Journal of Neural Transmission**, v. 104, n. 8-9, p. 953-966, 1997.

RÉNYI, L. The effect of selective 5-hydroxytryptamine uptake inhibitors on 5-methoxy-N, M-dimethyltryptamine-induced ejaculation in the rat. **Brazilian Journal of Pharmacology**, v. 87, n. 4, p. 639-648, 1986.

ROCHA, D. M. C.; DEBELJUK, L.; FRANÇA, L. R. Exposure to constant light during testis development increases daily sperm production in adult Wistar rats. **Tissue and Cell**, v. 31, n. 3, p. 372-379, 1999.

ROSEN, R. C.; LANE, R. M.; MENZA, M. Effects of SSRIs on sexual function: a critical review. **Journal of Clinical Psychopharmacology**, v. 19, n. 1, p. 67-85, 1999.

RUSSELL, L. D.; FRANÇA, L. R. Building a testis. **Tissue and Cell**, v. 27, n. 2, p. 129-147, 1995.

RUSSELL, D. L.; ETTLIN, R. A.; SINHA HIKIM, A. P.; CLEGG, E. D. Mammalian spermatogenesis. In: RUSSELL, D. L.; ETTLIN, R. A.; SINHA HIKIM, A. P.; CLEGG, E. D. (Ed.). **Histological and histopathological evaluation of the testis**. Bolesta: Cache River Press, 1990. Cap. 1, p. 1-40.

SILVA JUNIOR, V. A.; VIEIRA, A. C. S.; PINTO, C. F.; PAULA, T. A.; PALMA, M. B.; LINS AMORIM, M. J.; AMORIM JUNIOR, A. A.; MANHÃES-DE-CASTRO, R. Neonatal treatment with naloxone increases the population of Sertoli cells and sperm production in adult rats. **Reproduction Nutrition Development**, v. 46, n. 2, p. 157-166, 2006.

SILVA JUNIOR, V. A.; LINS, A. M. J. A. A.; AMORIM, J. A. A.; PINTO, C. F.; DEIRÓ, T. B. J.; OLIVEIRA, J. R. M.; PEIXOTO, C. A.; MANHÃES-DE-CASTRO, R. Neonatal administration of fluoxetine decreased final Sertoli cell number in Wistar rats. **International Journal of Morphology**, v. 26, n. 1, p. 51-62, 2008.

SIMANSKY, K. J. Serotonergic control of the organization of feeding and satiety. **Behav Brain Research**, v. 73, n. 1-2, p. 37-42, 1995.

TORRES, G.; HOROWITZ, J. M.; LAFLAMME, N.; RIVEST, S. Fluoxetine induces the transcription of genes encoding *c-fos*, corticotropin-releasing factor and its type 1 receptor in rat brain. **Neuroscience**, v. 87, n. 2, p. 463-477, 1998.

WALDINGER, M. D.; OLIVIER, B. Selective serotonin reuptake inhibitor-induced sexual dysfunction: clinical and research considerations. **International Clinical of Psychopharmacology**, v. 13, n. 6, p. 27-33, 1998.

Received on August 20, 2010.
Accepted on February 1, 2012.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.