Chemosphere 193 (2018) 958–967

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Atrazine affects the morphophysiology, tissue homeostasis and aromatase expression in the efferent ductules of adult rats with mild alterations in the ventral prostate

Elisângela Martins-Santos, Cristiano Guimarães Pimenta, Pollyana Rabelo Nunes Campos, André Gustavo Oliveira, Germán Arturo Bohórquez Mahecha, Cleida Aparecida Oliveira*

*Department of Morphology, Universidade Federal de Minas Gerais, Cx. Postal 486, CEP 31.270-901, Belo Horizonte, MG, Brazil*

## HIGHLIGHTS

- Atrazine alters efferent ductules morphology with mild effects on rat ventral prostate.
- Atrazine induces aromatase expression in the efferent ductules but not in the ventral prostate.
- Atrazine effects in testis may be secondary to alterations in the efferent ductules.

## ARTICLE INFO

*Article history:*
Received 5 September 2017
Received in revised form
20 November 2017
Accepted 22 November 2017
Available online 22 November 2017

Handling Editor: Shane Snyder

*Keywords:*
Atrazine
Efferent ductules
Aromatase
Endocrine disruptor
Prostate
Tissue homeostasis

## ABSTRACT

The widely used herbicide atrazine is a potent endocrine disruptor known to cause increased aromatase expression and transient increase in testicular weight followed by remarkable testis atrophy. However, whether the effects of atrazine on the testes are primary or secondary to dysfunctions in other components of male reproductive tract remains unknown. Given the high sensitivity of the efferent ductules to estrogen imbalance and the similarity to alterations previously described for other disruptors of these ductules function, and the testicular alterations observed after atrazine exposure, we hypothesized that the efferent ductules could be a target for atrazine. Herein we characterized the efferent ductules and the ventral prostate of adult Wistar rats treated with 200 mg/kg/day of atrazine for 7, 15, and 40 days. Additionally, we evaluated if the effects of atrazine in these organs could be reduced after discontinuation of the treatment. Atrazine exposure resulted in mild effects on the ventral prostate, but remarkable alterations on the efferent ductules, including luminal dilation, reduced epithelial height, and disruption of the epithelial homeostasis, which coincides with increased aromatase expression. Together with our previous data, these results suggest that at least part of the testicular effects of atrazine may be secondary to the alterations in the efferent ductules.



## 1. Introduction

Exposure to estrogenic or antiestrogenic compounds and impairment of estrogen receptors by genetic or chemical inactivation are among the main causes of male infertility and have been shown to cause major dysfunction of the efferent ductules (Hess et al., 1997a, 2000; Lee et al., 2001; Mckinnell et al., 2001; Oliveira et al., 2001, 2002; Zhou et al., 2001; Lee et al., 2009; Hess et al., 2011; Nanjappa et al., 2016). The efferent ductules are the segment of the male reproductive tract presenting the highest levels of estrogen in the luminal fluid and the highest expression of estrogen receptor in the epithelium (Hess et al., 1997b). Their main function is to reabsorb fluid coming from the testis, which occurs under the control of estrogens (Hess et al., 1997a, 2000; Lee et al., 2001; Oliveira et al., 2001, 2002, 2005; eira et al., 2002; Oliveira et al., 2005). Disturbance in fluid reabsorption leads to the accumulation of fluid in the ductule lumen and consequent reflux to the testis, thus resulting in luminal dilation of the seminiferous tubules followed by testicular atrophy and, consequently, infertility (Hess et al., 1997a; Oliveira et al., 2001; Oliveira et al., 2002).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine)

* Corresponding author. Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil.
*E-mail address:* cleida@icb.ufmg.br (C.A. Oliveira).

https://doi.org/10.1016/j.chemosphere.2017.11.124
0045-6535/

is a widely used herbicide shown to be a potent endocrine disruptor and cause adverse effects on the male genital system (Kniewald et al., 2000; Betancourt et al., 2006; Hayes et al., 2006, 2010; Swan, 2006; Suzawa and Ingraham, 2008; Rey et al., 2009; Belloni et al., 2011; Hayes et al., 2011). Potential risks for animal health include the increase in estrogen and reduction in the testosterone levels (Stoker et al., 2000; Friedmann, 2002; Victor-Costa et al., 2010). Among the effects of atrazine on male rats are testicular alterations, such as a transient increase in weight followed by a remarkable testicular atrophy (Victor-Costa et al., 2010), with a concurrent increase in aromatase immunoexpression in Leydig cells (Martins-Santos et al., 2017). Although evidence suggests that this key enzyme for estrogen production could be a target for the herbicide (Crain et al., 1997; Sanderson et al., 2000, 2001, 2002; Heneweer et al., 2004; Laville et al., 2006; Sanderson, 2006; Fan et al., 2007a, 2007b; Holloway et al., 2008; Tinfo et al., 2011), atrazine's mechanism of action remains to be elucidated. Moreover, most of the atrazine's effects on aromatase were demonstrated *in vitro* (Sanderson et al., 2000, 2001, 2002; Heneweer et al., 2004; Betancourt et al., 2006; Laville et al., 2006; Fan et al., 2007a, 2007b; Holloway et al., 2008; Suzawa and Ingraham, 2008; Higley et al., 2010; Tinfo et al., 2011; Quignot et al., 2012; Fa et al., 2013; Caron-Beaudoin et al., 2016).

One important question that remains unanswered is whether the atrazine effects in the testis are primary or secondary to changes in other testicular segments of the male reproductive tract. Given the high sensitivity of the efferent ductules to estrogen imbalance, the similarity to the alterations previously described for other disruptors of these ductules function (Nakai et al., 1992, 1993; Hess, 1998; Gotoh et al., 1999), and the testicular alterations observed after atrazine exposure, we hypothesized that the efferent ductules could be a target for atrazine. We investigated this hypothesis by evaluating the effects of atrazine on the morphology, aromatase expression, cell proliferation, and apoptosis of the efferent ductules. For comparison purposes, we also evaluated the effects of atrazine on the ventral prostate, which is an important target for androgens and estrogens (Ellem and Risbridger, 2010).

## 2. Materials and methods

### 2.1. Animals

Sexually mature male Wistar rats (100 days old), acquired from the Animal Facility at the Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Brazil, were used in this study. The rats were maintained under constant conditions of light (12/12 h light/dark cycles) and temperature (22 °C), receiving pelletized chow (Nuvital Nutrientes S.A., Colombo, PR, Brazil) and water *ad libitum*. All experimental procedures followed the guidelines of UFMG's animal care and research of the Institutional Ethical Committee on Animal Use (https://www.ufmg.br/bioetica/ceua/). The study was approved by the Institutional Ethical Committee for Animal Experimentation (Protocol number 287/2008).

### 2.2. Treatment

For 7, 15, and 40 days, sexually mature male Wistar rats received a daily dose of 200 mg/kg of atrazine (Gesaprim 500 Ciba Geigy, Syngenta, São Paulo, Brazil) diluted in corn oil. The control group received only corn oil. The dosages and treatment periods were based in previous studies showing alterations in testicular morphology and steroidogenesis (Victor-Costa et al., 2010; Martins-Santos et al., 2017).

In order to determine if the possible alterations caused by atrazine are permanent or transitory, after the 40-days treatment, a group of rats was maintained in the animal facility under the same environmental conditions for further 75 days (ATZ 40d Rec), without any treatment. Control of the recovery group (Cont 40d Rec) consisted of rats receiving only corn oil for 40 days and maintained for further 75 days (Martins-Santos et al., 2017).

### 2.3. Tissue preparation

Subsequently to each treatment and recovery periods, the rats were weighed, anesthetized with intraperitoneal injection of sodium pentobarbital (80 mg/kg) and ketamine chlorydrate (10 mg/kg) and then perfused intracardially with Ringer solution, followed by 10% (v/v) of neutral buffered formalin (NBF). After fixation, the efferent ductules and ventral prostate were dissected, immersed in NBF, and stored at 4 °C. Fragments of both segments were routinely processed for histological and immunohistochemical analyses.

### 2.4. Histology and morphometry

The tissue fragments were dehydrated with ascending concentrations of ethanol solutions, embedded in paraffin (Histosec pastilhas, Merck KGaA, Darmstadt, Germany), cut into 5-μm-thick sections and placed on glass slides. The sections were stained with hematoxylin and eosin (HE), and 1% toluidine blue-sodium borate solution for histological analysis.

The luminal diameters of the proximal and distal efferent ductules were measured at the widest region of five randomly chosen transversal sections per animal, by using a scale of 1000 μm coupled to the eyepiece of the microscope (Nikon Eclipse E200, Melville, USA). Measurements were made at 400X magnification.

The height of the proximal efferent ductules epithelium was measured from the basement membrane to the microvillus tip, in areas of straight sections from 25 cells with evident nuclei (in five tubule sections). Pictures were taken by using Pannoramic Viewer software (3DHISTECH Ltd., Budapest, Hungary). The proximal area was selected for this measurement based on previous studies showing that this is the region more sensitive to disruption on the estrogen responsive system (Oliveira et al., 2002).

### 2.5. Immunohistochemistry

Immunohistochemistry was performed to detect aromatase in the efferent ductules and the ventral prostate and to determine possible alterations in cell proliferation and apoptosis by using MCM7 and caspase-3 as markers, respectively.

To this end, fragments of NBF-fixed tissues were embedded in paraffin, cut into 5-μm-thick sections, mounted on glass slides, dewaxed, re-hydrated in graded ethanol, and incubated in methanol containing 0.6% $H_2O_2$ to inactivate the endogenous peroxidase. After microwaving in 0.1 M citrate buffer, pH 6.0, for antigen retrieval, the endogenous biotin activity was blocked by avidin and biotin-blocking solution (avidin/biotin blocking kit; Vector Laboratories, Burlingame, USA). To block non-specific antibody binding, the sections were incubated with 10% normal goat serum, followed by an overnight incubation at 4 °C with the primary antibodies: polyclonal rabbit anti-human aromatase (Sigma-Aldrich, Saint Louis, USA), diluted 1:500; monoclonal mouse *anti*-CDC47/MCM7 (Ab-2, Thermo Fisher Scientific, Rockford, USA), diluted 1:500; or polyclonal rabbit anti-cleaved caspase-3 (Millipore Corporation, California, USA), diluted 1:200. Negative controls were incubated with phosphate buffer saline (PBS) instead of the primary antibodies. Tissues were then incubated for 1 h with a biotinylated secondary antibody: goat anti-rabbit (for aromatase and caspase-3) or goat anti-mouse (for MCM7) (Dako, Carpinteria, USA), both diluted 1:100. This step was followed by incubation with avidin-

biotin complex conjugated with peroxidase (Vectastain Elite ABC kit – Vector Laboratories, Burlingame, USA), for 30 min. The immunoreaction was visualized using 3,3 diaminobenzidine containing 0.01% hydrogen peroxide in 0.05 M Tris-HCl buffer, pH 7.6. Sections were counterstained with Mayer's hematoxylin and mounted.

### 2.6. TUNEL

Apoptotic cells were also investigated in the efferent ductules by TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling), using the ApopTag Plus Peroxidase *In Situ* Apoptosis Detection Kit (Millipore Corporation, California, USA). The assays were performed according to the manufacturer's instructions, with some modifications described in (Gonzaga et al., 2017).

### 2.7. Quantitative analyses

The intensity of the cytoplasmic immunoreaction for aromatase in the epithelium was measured in five randomly chosen areas of the proximal and distal efferent ductules and the ventral prostate, for each animal, using the Adobe Photoshop CS6 (Adobe Systems Software, Mountain View, USA). The images were taken at 40X magnification, using the Pannoramic Viewer software (3DHISTECH Ltd., Budapest, Hungary), converted to grayscale mode and inverted. The average number of pixels was measured in the apical cytoplasm of six non-ciliated cells of the efferent ductules and luminal cells of the prostate per area, thus totalizing 30 cells per animal (Oliveira et al., 2007, 2013).

Metachromatic toluidine blue stained mast cells were counted in the stroma of the ventral prostate. The quantification was performed at 400X magnification in 10 different areas per animal. Results were expressed as number of mast cells per $mm^2$ (Martins-Santos et al., 2017).

The number of MCM7, caspase-3, and TUNEL positive cells were counted among 100 epithelial cells from five randomly chosen areas of the proximal and distal efferent ductules and the ventral prostate as in (Gonzaga et al., 2017). Positive and negative cells were counted on images taken at 400X magnification, using the Pannoramic Viewer software (3DHISTECH Ltd., Budapest, Hungary). The results were expressed as percentage of positive epithelial cells.

### 2.8. Statistical analysis

Data obtained from quantitative studies were analyzed using GraphPad Prism (GraphPad Software, San Diego, California, USA). The data were submitted to Shapiro-Wilk normality test and investigated for multiple variances using one-way ANOVA and a post-hoc Tukey's test to compare more than two populations or Student's t-test was used to compare the means between two groups. Nonparametric data were analyzed using the Mann-Whitney and Kruskal-Wallis followed by Dunn's post-hoc tests for comparisons between two or more groups, respectively. The data were expressed as the mean ± SEM, and differences were considered significant at $p \leq 0.05$.

## 3. Results

### 3.1. Atrazine treatment alters efferent ductules histology with mild effects on the ventral prostate of male rats

After 7 days of treatment, the morphology of the efferent ductules from rats exposed to atrazine was similar to the controls (Fig. 1), except for a slight decrease in epithelial height (Fig. 1S). After 15 and 40 days of atrazine exposure, the rats exhibited luminal dilation (Fig. 1) and further reduction in epithelial height in most efferent ductules (Fig. 1S). The luminal dilation increased 58% and 34% in the proximal ductules and 119% and 57% in the distal ductules after 15 and 40 days of treatment, respectively (Fig. 1T–U). The epithelial height reduced 50% and 54% in the proximal ductules after 15 and 40 days of treatment, respectively (Fig. 1Q–S). After 40 days, we also observed luminal sloughed epithelial cells (insert in Fig. 1O).

On the other hand, the luminal diameter of proximal and distal ductules significantly decreased (61% and 18%, respectively) after the recovery period (Fig. 1T–U). We also observed a 15% reduction in the epithelial height of the proximal efferent ductules of rats of the recovery group in comparison with the control (Cont 40d Rec) (Fig. 1S).

The morphology of the ventral prostate varied considerably among the animals after atrazine exposure and for each period of treatment, since we observed glands with normal appearance and glands with the presence of cystic or hyperplasic acini. Foci of inflammatory cells were frequent in the stroma, especially after long-term treatment (40 days), and were still present after the recovery period (Fig. 2A–F). Accordingly, the number of metachromatic mast cells were similar when treated and control tissue were compared (Fig. 2G). Considering the heterogeneity of the prostate tissue among treated animals, we analyzed prostate tissue of another group of rats exposed to atrazine for 40 days and the respective control ($n = 5$), confirming the results. The prostate appeared mostly normal even in animals with remarkable changes in testis morphology (Fig. 2H), as previously shown (Martins-Santos et al., 2017).

### 3.2. Aromatase expression is altered in the efferent ductules but not in the ventral prostate following atrazine treatment of male rats

Aromatase was immunodetected in the cytoplasm of non-ciliated epithelial cells of the efferent ductules in all the animals, whereas staining of ciliated cells was intermittent (Fig. 3). The groups treated with atrazine, including the recovery group (ATZ 40d Rec), presented increased cytoplasmic aromatase staining at both proximal and distal regions of the efferent ductules in comparison with the controls (Fig. 3).

Immunoreaction was positive for aromatase in the cytoplasm of the luminal cells of the ventral prostate, intermittent in the basal cells, and not detected in the peritubular smooth muscle cells (Fig. 4A–E). No detectable changes were observed in the pattern and intensity of the aromatase immunoreactivity in the luminal cells after atrazine treatment (Fig. 4F).

### 3.3. Cell proliferation increases in efferent ductules and decreases in the ventral prostate of atrazine-treated male rats

Cell proliferation in the efferent ductules and ventral prostate was assessed using the MCM7 marker. In control animals, the protein was detected in the nuclei of some cells along the efferent ductules epithelium, mainly in non-ciliated cells (Fig. 5). Cells were found more frequently dividing perpendicular to the basement membrane (Fig. 5C) or in small groups (Fig. 5K), whereas some cells were found dividing parallel to the basement membrane (Fig. 5J). After exposure to atrazine, cell proliferation increased (Table 1). The pattern of cell division was similar to that found in control groups, which also showed cellular divisions parallel and perpendicular to the basement membrane. Many cells were dividing in parallel, thus resulting in rows of MCM7 positive cells. In the recovery groups, we observed few cells positive for MCM7 in atrazine-treated and control animals, in both the proximal (Fig. 5 D and H) and distal regions (Fig. 5 L and P) of the efferent ductules.

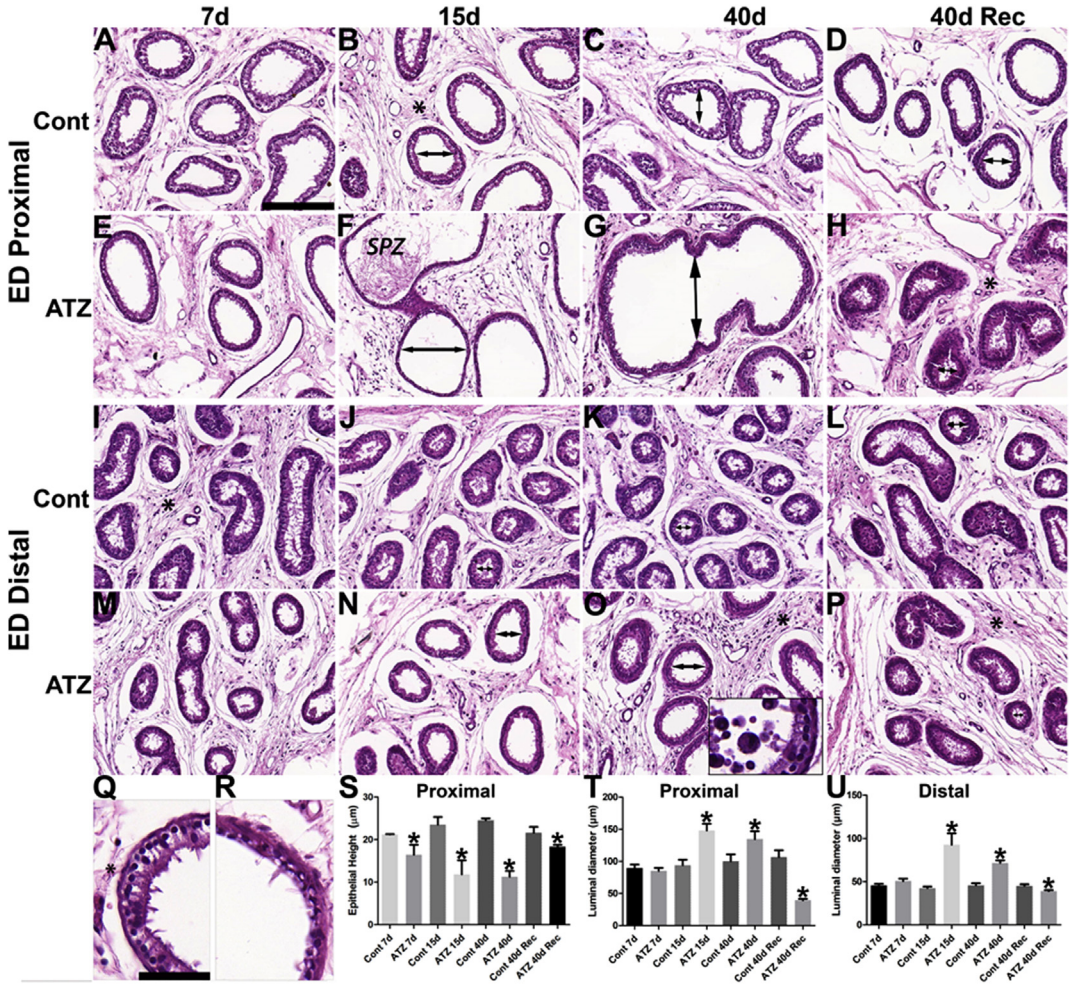


**Fig. 1.** Effects of atrazine on the morphology of the efferent ductules of Wistar rats. **(A - D, I - L)** Representative efferent ductules of control animals. (**E - G, M - O)** Efferent ductules of rats treated with atrazine (200 mg/kg/day) for 7, 15, and 40 consecutive days, respectively. **(H and P)** ED of rats treated with atrazine for 40 days followed by a 75-day recovery period (ATZ 40d Rec). **(Q, R and S)** Effects of atrazine on the epithelial height of efferent ductules: **(Q)** normal epithelium; **(R)** reduced epithelial height; **(S)** Graphical representation of the epithelial height of the proximal efferent ductules. **(T and U)** Graphical representation of effects of atrazine on luminal diameter of the **(T)** proximal and **(U)** distal efferent ductules. Scale bar in A (= B to P) = 200 μm; Scale bar in Q (= R) = 50 μm; Double arrows indicate luminal diameter; * = stroma; *SPZ* = sperm; Insert in O indicate sloughed epithelial cells; Cont = Control; ATZ = atrazine; Rec = recovery group; d = days of treatment; n = 4; *P ≤ 0.05 compared to the respective control.

Considering the scarcity of information regarding cell proliferation in the normal efferent ductules, we compared the proliferation rate from control rats at different ages (107, 115, 140 and 210 days old). We detected a gradual decrease in cell proliferation with aging, which was statistically significant at the proximal and distal efferent ductules (Table 2). When the proliferation rates of proximal versus distal region were compared, we observed a higher proliferation rate at the distal efferent ductules at all time points analyzed (Table 2).

In atrazine-exposed rats, cell proliferation was also reduced in the ventral prostate epithelium. The index of MCM7 positive cells was smaller in the gland of treated animals when compared with the controls, with rates of 85.6%, 90.8%, and 86.5% at 7, 15, and 40 days of exposure, respectively (Table 1). After the recovery period, cell proliferation in the ventral prostate of atrazine-treated animals (ATZ 40d Rec) returned to normal levels.

### *3.4. Apoptosis increases in the distal efferent ductules of atrazine-treated male rats and in the ventral prostate of animals subjected to a recovery period*

Apoptosis in the efferent ductules was assessed using the marker caspase-3 and TUNEL. Caspase-3-positive cells were undetectable in the epithelium of the proximal ductules and scarce (0.02%) in the distal ductules of control animals (Table 1). After exposure to atrazine, the amount of caspase-3 positive epithelial cells was variable, as in some animals these cells were undetectable whereas in others they appeared increased, especially in the distal ductules. As a result of this heterogeneity, statistical significance was reached just for distal efferent ductules of animals treated for 40 days, even though the number of cells was 4–4.5 times greater in the treated group. After recovery, the number of caspase-3 positive cells was similar when ductules from treated and control rats, at both proximal and distal regions, were compared.

The distribution pattern of TUNEL-positive cells was similar to that observed for caspase-3. After all treatment periods, a trend towards increased positivity was observed in both the proximal and the distal efferent ductules and reached statistical significance after 40 days of exposure (Table 1). The percentage of TUNEL-positive cells was similar in the recovery groups of atrazine-treated and control animals.

Caspase-3-positive cells were rare or undetectable in the epithelium of the ventral prostate of control and treated animals, except in the recovery group (ATZ 40d Rec), in which a significant

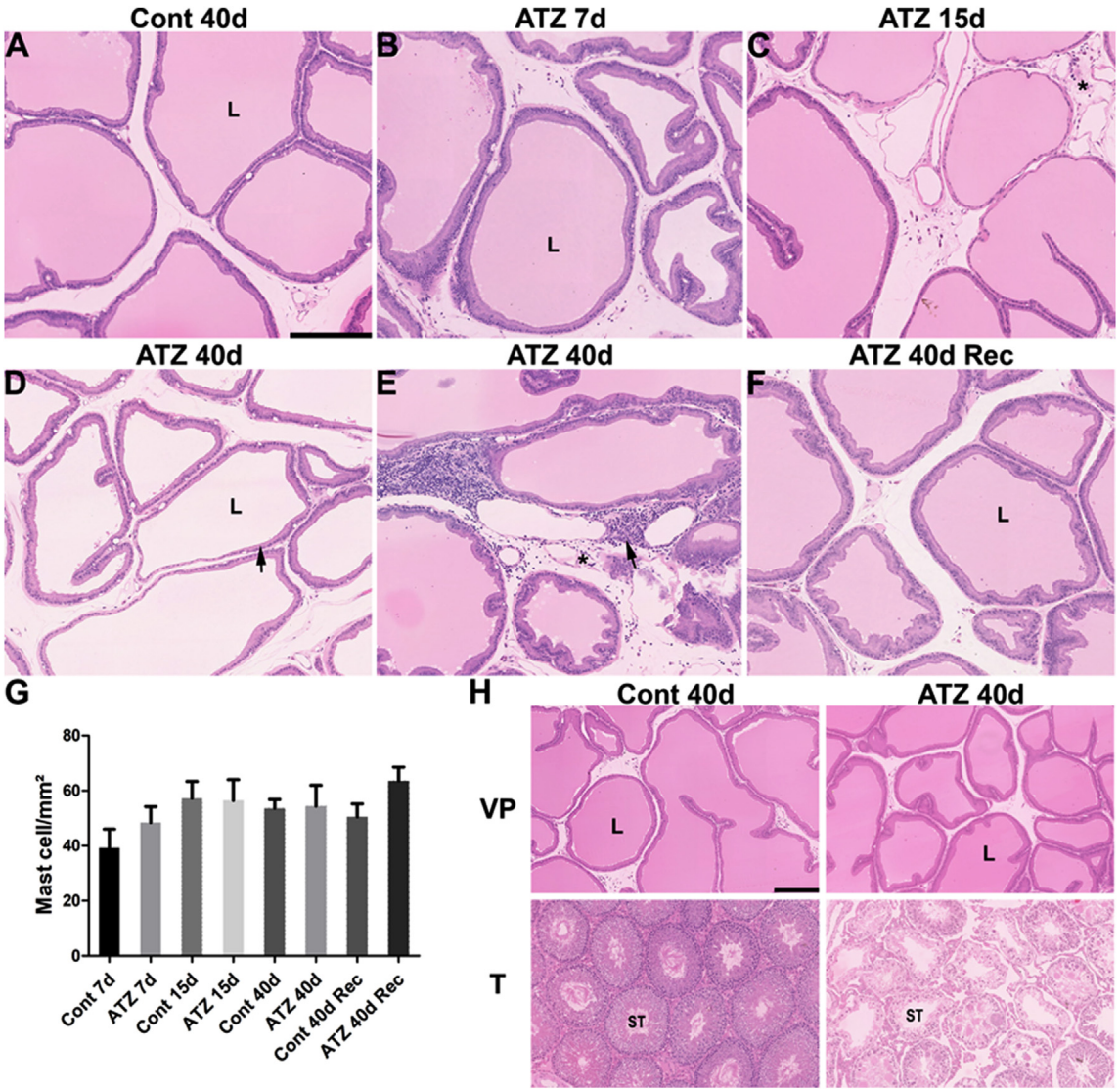


**Fig. 2.** Effects of Atrazine on morphology of the ventral prostate of Wistar rat. **(A)** Representative image of the ventral prostate of control animals. **(B, C, D and E)** Ventral prostate of rats treated with atrazine 200 mg/kg/day for 7, 15, and 40 days, respectively; **(D)** normal ventral prostate and **(E)** ventral prostate with inflammatory foci after atrazine exposure. **(F)** Ventral prostate of rats treated with atrazine for 40 days followed by a 75-day recovery period (ATZ 40d Rec). **(G)** Number of metachromatic mast cells per $mm^2$ in the stroma of ventral prostates. **(H)** Representative images of mild effects on ventral prostate even in animals with remarkable testicular changes, compared to control. n = 4. Scale bar = 200 μm; * = stroma; L = lumen; arrow = inflammatory foci; VP = ventral prostate; T = testis.

increase of caspase-3-positive cells was observed when compared to the respective control (Table 1).

## 4. Discussion

In the present study, we investigated whether the atrazine effects previously observed in the testis (Victor-Costa et al., 2010; Martins-Santos et al., 2017) were primary or secondary to changes in other male reproductive tract segments. We show that exposure to atrazine results in increased luminal diameter, reduced epithelial height, increased aromatase expression, and disrupted rates of cell proliferation and apoptosis in the rat efferent ductules, whereas the effects on the ventral prostate were mild. Given that similar alterations in the efferent ductules lead to dilation and testicular atrophy (Hess et al., 1997a; Oliveira et al., 2001; Oliveira et al., 2002), our results favor the hypothesis that the testicular changes in rats exposed to atrazine may be, at least in part, secondary to the alterations in the efferent ductules.

The significant increase observed in the luminal diameter and the reduction in the epithelial height closely resemble those associated with impairment in luminal fluid reabsorption in rodent efferent ductules induced by alteration in the estrogen responsive system (Hess et al., 1997a; Oliveira et al., 2001; Zhou et al., 2001; Oliveira et al., 2002; Cho et al., 2003; Oliveira et al., 2003; Nanjappa et al., 2016). Non-reabsorbed fluid accumulates in the lumen and returns to the testis, ultimately causing testicular damages, such as seminiferous tubules dilation and atrophy, followed by a reduction in sperm and infertility (Hess et al., 1997a; Oliveira et al., 2001; Oliveira et al., 2002). Our present and previous results (Victor-Costa et al., 2010; Martins-Santos et al., 2017) suggest that similar mechanism may explain the effects observed in the efferent ductules and testis following exposure to atrazine. Interestingly, after the recovery period, the luminal diameter of the efferent ductules was greatly reduced when compared to controls. Although there was a clear trend for recovery of the epithelial height, it remained lower than controls after recovery. Reduction in

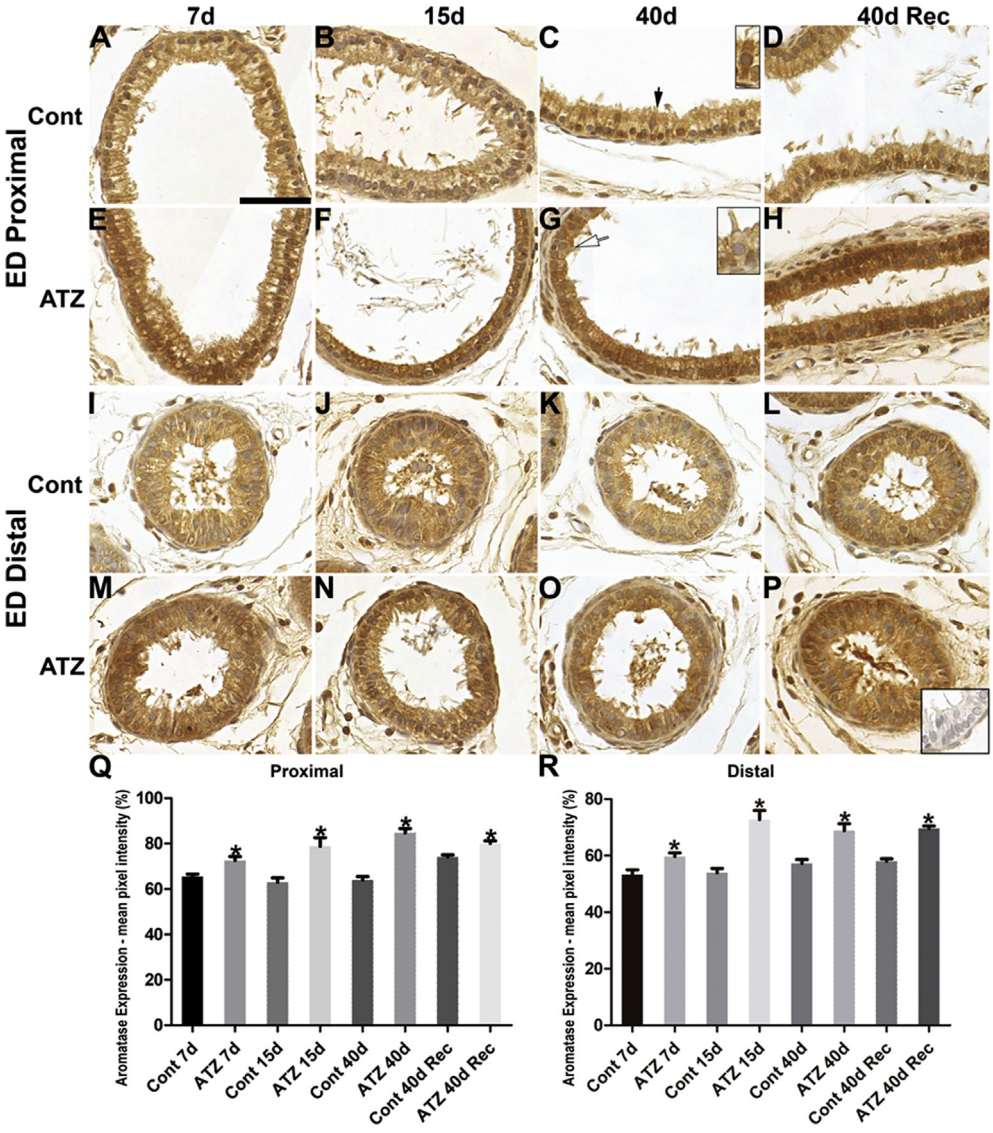


**Fig. 3.** Effect of atrazine on expression of aromatase in the efferent ductules of Wistar rats. **(A - D, I - L)** Representative efferent ductules of control animals. **(E - G, M - O)** Efferent ductules of rats treated with atrazine (200 mg/kg/day) for 7, 15, and 40 consecutive days, respectively. **(H and P)** Efferent ductules of rats treated with atrazine for 40 days followed by a 75-day recovery period (ATZ 40d Rec). **(Q and R)** Graphical representation of the quantification of aromatase expression in proximal **(Q)** and distal **(R)** efferent ductules. Scale bar in A = 50 μm; Insert in P = negative control; Arrow and insert in C = positive ciliated cells; hollow arrow and insert in G = Ciliated negative cells; ED = Efferent ductules; Cont = Control; ATZ = atrazine; Rec = recovery group; d = days of treatment; n = 4; *P ≤ 0.05 compared to the respective control.

the diameter and epithelial height of the efferent ductules coincides with testis atrophy (Martins-Santos et al., 2017), thus emphasizing the intricate relationship between testis and efferent ductules.

The morphological changes promoted by the herbicide in the efferent ductules were similar to those found in other experimental models of androgen and estrogen imbalance (Mckinnell et al., 2001; Oliveira et al., 2001, 2002; Cho et al., 2003). Stimulation of aromatase has long been speculated as a possible mechanism of atrazine action on the male reproductive system (Crain et al., 1997; Sanderson et al., 2000, 2001; Hayes et al., 2002; Sanderson et al., 2002; Heneweer et al., 2004; Hayes et al., 2006; Laville et al., 2006; Sanderson, 2006; Fan et al., 2007a, 2007b; Holloway et al., 2008; Tinfo et al., 2011; Quignot et al., 2012; Jin et al., 2013; Thibeault et al., 2014; Caron-Beaudoin et al., 2016; Martins-Santos et al., 2017). Indeed, we found a remarkable increase in aromatase immunoreactivity in the efferent ductules after atrazine exposure. This increase was detected as early as 7 days after the treatment and remained high even after the recovery period, in contrast to the testis, which showed aromatase expression increase only after 40 days of exposure (Martins-Santos et al., 2017). These data suggest that aromatase may be a direct target of atrazine on the efferent ductules.

Since the cell proliferation profile has not been previously established in the epithelium of rodent efferent ductules, we first characterized MCM7 expression under normal conditions. Few proliferative cells were observed in the efferent ductules of adult rats and they were mainly non-ciliated cells, thus confirming that this is a stable tissue during adulthood. Cell division occurred both parallel and perpendicular to the basement membrane. In the epididymal duct, these patterns of division are known to result in increased duct length and diameter, respectively (Hinton et al., 2011). We observed regional variations in cell proliferation, as higher rates were found in the distal efferent ductules when

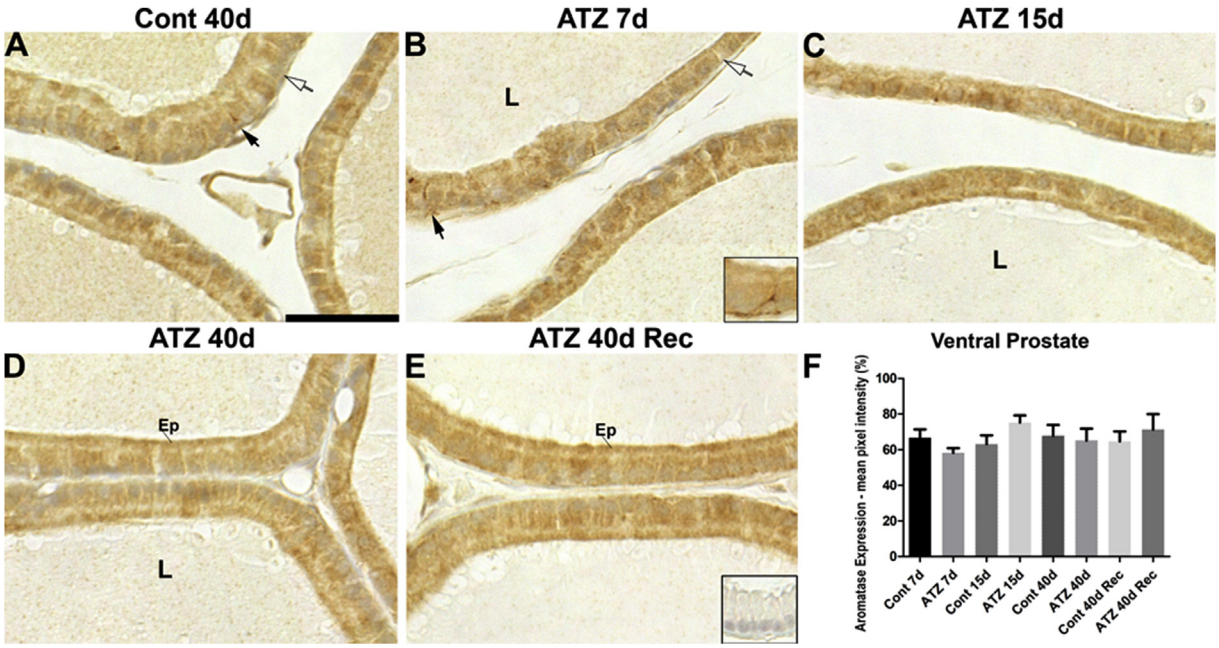


**Fig. 4.** Effects of atrazine on aromatase expression in the ventral prostate of Wistar rat. **(A)** Representative image of the ventral prostate of the control animals. **(B–D)** Ventral prostate of rats treated with atrazine 200 mg/kg/day for 7, 15, and 40 days, respectively. **(E)** Ventral prostate of rats treated with atrazine for 40 days followed by a 75-day recovery period (ATZ 40d Rec). **(F)** Graphical representation of quantification of aromatase expression in the ventral prostate. Scale bar in A = 50 ìm; Insert in B and arrow = positive basal cell; hollow arrow = negative basal cell; Insert in E = negative control; L = lumen; Ep = epithelium positive for aromatase. n = 4; *P ≤ 0.05 compared to the respective control.

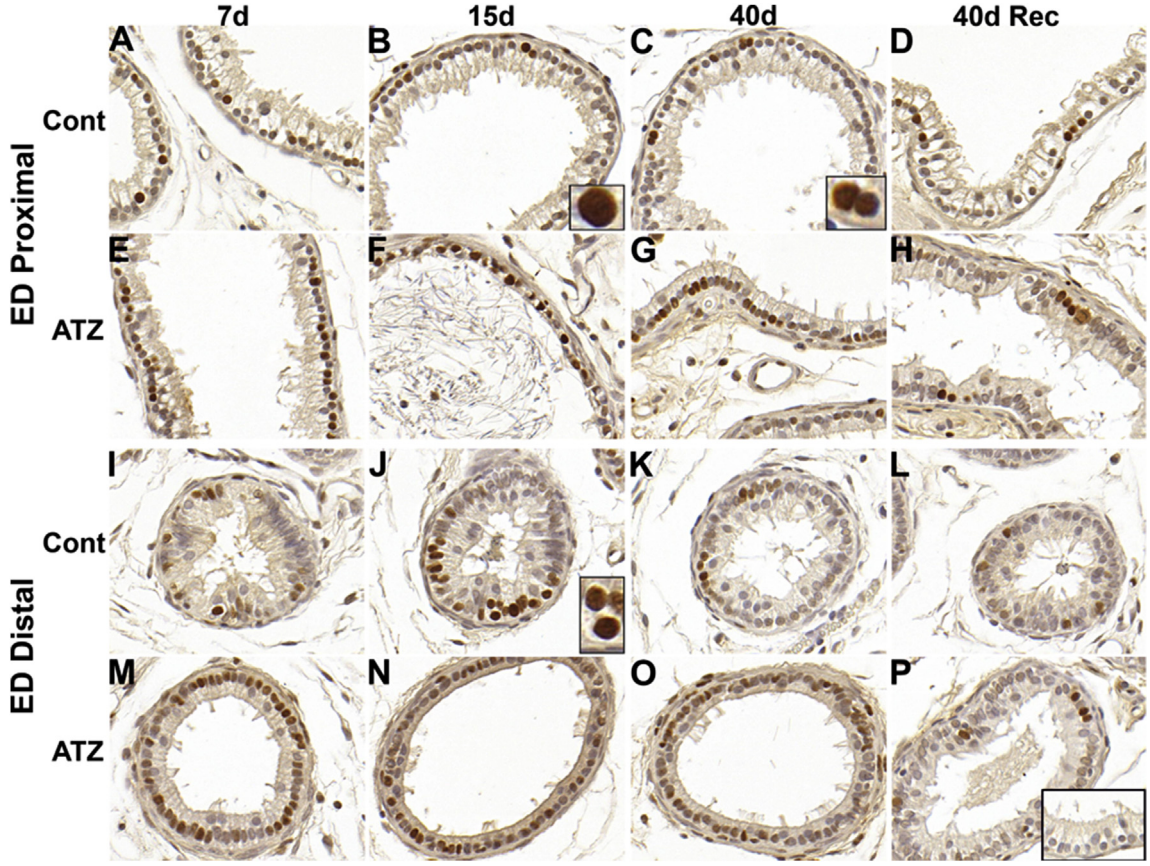


**Fig. 5.** Effects of atrazine on proliferative MCM7 positive cells in the efferent ductules of Wistar rat. **(A - D, I - L)** Representative efferent ductules (ED) of control animals. **(E - G, M - O)** ED of rats treated with atrazine (200 mg/kg) for 7, 15 and 40 consecutive days, respectively. **(H and P)** ED of rats treated with atrazine for 40 days followed by a 75-day recovery period (ATZ 40d Rec). Scale bar in A = 50 μm; Insert in B = detail of a positive nuclei; Insert in C = cells dividing perpendicular to the basement membrane; Insert in J = cells dividing parallel to the basement membrane; Insert in P = negative control; Arrowheads = positive cells; Cont = Control; ATZ = atrazine; Rec = recovery; d = days.

**Table 1**
Effect of atrazine on cell proliferation and apoptosis in the ventral prostate and efferent ductules of adult male Wistar rats.

| | Cont 7d | ATZ 7d | Cont 15d | ATZ 15d | Cont 40d | ATZ 40d | Cont 40d Rec | ATZ 40d Rec |
|---|---|---|---|---|---|---|---|---|
| **MCM7** | 2010 | 2960* | 1680 | 4090* | 0,660 | 5830* | 0,810 | 1500 |
| ED proximal | ±1237 | ±1825 | ±0,834 | ±3149 | ±0,486 | ±2630 | ±0,375 | ±1439 |
| ED distal | 3470 | 7200* | 2420 | 3510* | 2480 | 4770* | 1440 | 1900 |
| | ±1484 | ±4076 | ±1214 | ±1905 | ±1016 | ±1779 | ±0,764 | ±1158 |
| VP | 31.67 | 4.54* | 28.90 | 2.64* | 13.87 | 1.87* | 3.40 | 5.93 |
| | ±20.42 | ±2.55 | ±19.35 | ±2.01 | ±5.29 | ±1.29 | ±2.60 | ±4.49 |
| **Caspase-3** | 0.00 | 0.02 | 0.00 | 0.04 | 0.00 | 0.02 | 0.00 | 0.01 |
| ED proximal | ±0.00 | ±0.04 | ±0.00 | ±0.08 | ±0.00 | ±0.02 | ±0.00 | ±0.02 |
| ED distal | 0.02 | 0.08 | 0.02 | 0.09 | 0.00 | 0.09* | 0.02 | 0.02 |
| | ±0.04 | ±0.07 | ±0.04 | ±0.13 | ±0.00 | ±0.08 | ±0.04 | ±0.02 |
| VP | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.12* |
| | ±0.00 | ±0.02 | ±0.00 | ±0.00 | ±0.00 | ±0.04 | ±0.00 | ±0.17 |
| **TUNEL** | 0.00 | 0.03 | 0.01 | 0.11 | 0.01 | 0.12* | 0.00 | 0.01 |
| ED proximal | ±0.00 | ±0.05 | ±0.02 | ±0.12 | ±0.02 | ±0.04 | ±0.00 | ±0.02 |
| ED distal | 0.04 | 0.07 | 0.00 | 0.08 | 0.00 | 0.05* | 0.00 | 0.01 |
| | ±0.04 | ±0.12 | ±0.00 | ±0.14 | ±0.00 | ±0.02 | ±0.00 | ±0.02 |

Results are presented as mean ± SEM. *P ≤ 0.05 compared to the respective control. Cont = Control; ATZ = atrazine; Rec = recovery; d = days; n = 3–4; ED = efferent ductules; VP = ventral prostate.

**Table 2**
Quantification of MCM-7 positive cells in the control proximal versus distal efferent ductules (ED) at different ages in the adult male Wistar rats.

| MCM7 | 107 days | 115 days | 140 days | 215 days |
|---|---|---|---|---|
| ED Proximal | 2,010[a] | 1,680[a] | 0,660[b] | 0,810[b] |
| | ±1237 | ±0,834 | ±0,486 | ±0,375 |
| ED Distal | 3,470[a*] | 2,420[a*] | 2,480[a*] | 1,440[b*] |
| | ±1484 | ±1214 | ±1016 | ±0,764 |

Results are presented as mean ± SEM. Different letters indicate P ≤ 0.05 among ages in the same region. *P ≤ 0.05 compared to respective age (proximal versus distal). n = 4.

compared to the proximal. These results are in agreement with data showing that expression of the androgen receptor (AR) and the estrogen receptor ERα, two potent proliferation factors, is higher in the distal efferent ductules (Oliveira et al., 2003). Additionally, we showed that the proportion of cell proliferation significantly decreased in the efferent ductules of older compared to younger animals. Although age-related changes in cell proliferation were still uncharacterized in the efferent ductules, similar morphological alterations and decreased proliferative activity have been previously reported in other segments of the male reproductive tract, such as the epididymis (Clermont and Flannery, 1970; Calvo et al., 1999).

After exposure to atrazine, we observed increased cell proliferation in the proximal and distal efferent ductules in comparison with the control at the same time points. In contrast, apoptosis remained unaffected in the experimental groups, except after 40 days of treatment, when apoptosis was higher in the treated animals, especially in the distal ductules. This disruption in the rates of cell proliferation and apoptosis after atrazine exposure corroborates other findings that this herbicide may affect tissue homeostasis in vertebrate species (Liu et al., 2006; Lenkowski et al., 2008; Rey et al., 2009; Zhang et al., 2011; Song et al., 2015) and suggests that this may be another possible mechanism of action for atrazine on animal tissues. Furthermore, cell proliferation and apoptosis in the recovery group (ATZ 40d Rec) returned to levels similar to the controls, thus indicating that the discontinuation of atrazine treatment was sufficient to re-establish tissue homeostasis.

Atrazine effects in male extragonadal organs, such as the prostate, are not well established. Herein, we showed variable changes in the morphology of the ventral prostate, especially regarding inflammation in the stroma after long-term exposure. These results are in agreement with previous data showing that atrazine acts as a prostatitis inductor in male rats (Stoker et al., 1999; Stanko et al., 2010). Atrazine exposure also reduced the prostate proliferation index in all the experimental groups, further emphasizing the above-mentioned capability of this herbicide to disrupt tissue homeostasis and its potential ability to promote tumor malignancy in the prostate (Hu et al., 2016). Following the cessation of exposure, cell proliferation returned to levels similar to the controls, suggesting that the effects may be at least partially reversible.

It is known that atrazine may be an inductor of aromatase in several human cancer cell lines (Sanderson et al., 2000, 2001, 2002; Heneweer et al., 2004; Fan et al., 2007a, 2007b; Tinfo et al., 2011; Quignot et al., 2012; Thibeault et al., 2014; Caron-Beaudoin et al., 2016), and correlated with an elevated risk of prostate cancer development (Hu et al., 2016). Surprisingly, we found that expression of this enzyme was not altered in the ventral prostates of rats exposed to atrazine, indicating that aromatase induction may be restricted to the efferent ductules and testis (Martins-Santos et al., 2017). Aromatase is knowingly tissue-specifically regulated (Mahendroo et al., 1993; Simpson and Davis, 2001). Likewise, atrazine induction of aromatase is also tissue-specific and appears to involve direct binding to the steroidogenic factor 1 (SF-1) (Fan et al., 2007a, 2007b; Suzawa and Ingraham, 2008). In this sense, tissues expressing SF-1 may be more susceptible to atrazine, which could explain the tissue-specific effects observed on aromatase induction, as SF1 is expressed in the testis and epididymis (Pezzi et al., 2004; Rivest et al., 2010), but is lacking in the normal prostate tissue (Lewis et al., 2014). Although this may offer a plausible explanation for the variable responses to the herbicide, further studies would be required to confirm this hypothesis.

## 5. Conclusion

In summary, this study revealed that atrazine effects on the ventral prostate are mild, but exposure to this herbicide leads to remarkable alterations on the efferent ductules, including luminal dilation and disruption of the epithelial homeostasis, which coincides with increased aromatase expression. Together with our previous data (Victor-Costa et al., 2010; Martins-Santos et al., 2017), these results suggest that at least part of the testicular effects of atrazine may be secondary to the alterations in the efferent ductules.

## Acknowledgements

This work was supported by the Conselho Nacional de

Desenvolvimento Cientifico e Tecnológico (CNPq) by means of a research fellowship and a research grant awarded to C.A.O., and a PIBIC scholarship to P.R.N.C. A master and doctoral fellowship was awarded to E.M.S by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) granted a PROBIC scholarship to C.G.P. The authors would like to thank the Centro de Aquisição e Processamento de Imagens (CAPI) of the Instituto de Ciências Biológicas of UFMG for providing the equipment and technical support for the experiments involving microscopic data. This manuscript was reviewed by a professional science editor and by a native English-speaking copy editor to improve readability, sponsored by the Pró-Reitoria de Pesquisa – PRPq/UFMG.

## References

Belloni, V., et al., Jan 11 2011. Early exposure to low doses of atrazine affects behavior in juvenile and adult CD1 mice. Toxicology 279 (1–3), 19–26. ISSN 1879-3185 (Electronic), 0300-483X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/20624442.

Betancourt, M., Resendiz, A., Fierro, E.C., Oct 2006. Effect of two insecticides and two herbicides on the porcine sperm motility patterns using computer-assisted semen analysis (CASA) in vitro. Reprod. Toxicol. 22 (3), 508–512. ISSN 0890-6238 (Print), 0890-6238 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16713176.

Calvo, A., et al., Dec 01 1999. Age-related changes in the hamster epididymis. Anat. Rec. 256 (4), 335–346. ISSN 0003-276X (Print), 0003-276X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10589020.

Caron-Beaudoin, E., Denison, M.S., Sanderson, J.T., Jan 2016. Effects of neonicotinoids on promoter-specific expression and activity of aromatase (CYP19) in human adrenocortical carcinoma (H295R) and primary umbilical vein endothelial (HUVEC) cells. Toxicol. Sci. 149 (1), 134–144. ISSN 1096-0929 (Electronic), 1096-0929 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/26464060.

Cho, H.W., et al., Aug 18 2003. The antiestrogen ICI 182,780 induces early effects on the adult male mouse reproductive tract and long-term decreased fertility without testicular atrophy. Reprod. Biol. Endocrinol. 1, 57. ISSN 1477-7827 (Electronic), 1477-7827 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/12959643.

Clermont, Y., Flannery, J., Dec 1970. Mitotic activity in the epithelium of the epididymis in young and old adult rats. Biol. Reprod. 3 (3), 283–292. ISSN 0006-3363 (Print), 0006-3363 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/5522858.

Crain, D.A., et al., May 1997. Alterations in steroidogenesis in alligators (Alligator mississippiensis) exposed naturally and experimentally to environmental contaminants. Environ. Health Perspect. 105 (5), 528–533. ISSN 0091-6765 (Print), 0091-6765 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/9222139.

Ellem, S.J., Risbridger, G.P., Feb 28 2010. Aromatase and regulating the estrogen: androgen ratio in the prostate gland. J. Steroid Biochem. Mol. Biol. 118 (4–5), 246–251. ISSN 1879-1220 (Electronic), 0960-0760 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/19896534.

Fa, S., et al., Jul 01 2013. Involvement of ERK1/2 signaling pathway in atrazine action on FSH-stimulated LHR and CYP19A1 expression in rat granulosa cells. Toxicol. Appl. Pharmacol. 270 (1), 1–8. ISSN 1096-0333 (Electronic), 0041-008X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/23583632.

Fan, W., et al., Apr 20 2007b. Herbicide atrazine activates SF-1 by direct affinity and concomitant co-activators recruitments to induce aromatase expression via promoter II. Biochem. Biophys. Res. Commun. 355 (4), 1012–1018. ISSN 0006-291X (Print), 0006-291X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/17331471.

Fan, W., et al., May 2007a. Atrazine-induced aromatase expression is SF-1 dependent: implications for endocrine disruption in wildlife and reproductive cancers in humans. Environ. Health Perspect. 115 (5), 720–727. ISSN 0091-6765 (Print), 0091-6765 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17520059.

Friedmann, A.S., May-Jun 2002. Atrazine inhibition of testosterone production in rat males following peripubertal exposure. Reprod. Toxicol. 16 (3), 275–279. ISSN 0890-6238 (Print), 0890-6238 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/12128101.

Gonzaga, A.C.R., et al., Jun 2017. Profile of cell proliferation and apoptosis activated by the intrinsic and extrinsic pathways in the prostate of aging rats. Prostate 77 (9), 937–948. ISSN 1097-0045 (Electronic), 0270-4137 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/28480526.

Gotoh, Y., et al., Jul 1999. Testicular damage after exposure to carbendazim depends on the number of patent efferent ductules. J. Vet. Med. Sci. 61 (7), 755–760. ISSN 0916-7250 (Print), 0916-7250 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10458097.

Hayes, T.B., et al., Oct 2011. Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. J. Steroid Biochem. Mol. Biol. 127 (1–2), 64–73. ISSN 1879-1220 (Electronic), 0960-0760 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21419222.

Hayes, T.B., et al., Apr 16 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc Natl Acad Sci U S A 99 (8), 5476–5480. ISSN 0027-8424 (Print), 0027-8424 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11960004.

Hayes, T.B., et al., Mar 9 2010. Atrazine induces complete feminization and chemical castration in male African clawed frogs (Xenopus laevis). Proc. Natl. Acad. Sci. U. S. A. 107 (10), 4612–4617. ISSN 1091-6490 (Electronic), 0027-8424 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20194757.

Hayes, T.B., et al., Apr 2006. Characterization of atrazine-induced gonadal malformations in African clawed frogs (Xenopus laevis) and comparisons with effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (17beta-estradiol): support for the demasculinization/feminization hypothesis. Environ. Health Perspect. 114 (Suppl. 1), 134–141. ISSN 0091-6765 (Print), 0091-6765 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/16818259.

Heneweer, M., Van Den Berg, M., Sanderson, J.T., Jan 15 2004. A comparison of human H295R and rat R2C cell lines as in vitro screening tools for effects on aromatase. Toxicol. Lett. 146 (2), 183–194. ISSN 0378-4274 (Print), 0378-4274 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/14643970.

Hess, R.A., 1998. Effects of environmental toxicants on the efferent ducts, epididymis and fertility. J. Reprod. Fertil. Suppl. 53, 247–259. ISSN 0449-3087 (Print), 0449-3087 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10645284.

Hess, R.A., et al., Dec 4 1997a. A role for oestrogens in the male reproductive system. Nature 390 (6659), 509–512. ISSN 0028-0836 (Print), 0028-0836 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9393999.

Hess, R.A., et al., Jan-Feb 2000. Morphologic changes in efferent ductules and epididymis in estrogen receptor-alpha knockout mice. J. Androl. 21 (1), 107–121. ISSN 0196-3635 (Print), 0196-3635 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10670526.

Hess, R.A., et al., Nov-Dec 2011. Estrogen and its receptors in efferent ductules and epididymis. J. Androl. 32 (6), 600–613. ISSN 1939-4640 (Electronic), 0196-3635 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/21441425.

Hess, R.A., et al., Nov-Dec 1997b. Estrogen receptor (alpha and beta) expression in the excurrent ducts of the adult male rat reproductive tract. J. Androl. 18 (6), 602–611. ISSN 0196-3635 (Print), 0196-3635 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/9432133.

Higley, E.B., et al., Jun 2010. Assessment of chemical effects on aromatase activity using the H295R cell line. Environ. Sci. Pollut. Res. Int. 17 (5), 1137–1148. ISSN 1614-7499 (Electronic), 0944-1344 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/20087668.

Hinton, B.T., et al., Nov-Dec 2011. How do you get six meters of epididymis inside a human scrotum? J. Androl. 32 (6), 558–564. ISSN 1939-4640 (Electronic), 0196-3635 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/21441421.

Holloway, A.C., et al., Apr 2008. Atrazine-induced changes in aromatase activity in estrogen sensitive target tissues. J. Appl. Toxicol. 28 (3), 260–270. ISSN 0260-437X (Print), 0260-437X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/17685393.

Hu, K., et al., May 2016. Atrazine promotes RM1 prostate cancer cell proliferation by activating STAT3 signaling. Int. J. Oncol. 48 (5), 2166–2174. ISSN 1791-2423 (Electronic), 1019-6439 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/26984284.

Jin, Y., Wang, L., Fu, Z., Apr 1 2013. Oral exposure to atrazine modulates hormone synthesis and the transcription of steroidogenic genes in male peripubertal mice. Gen. Comp. Endocrinol. 184, 120–127. ISSN 1095-6840 (Electronic), 0016-6480 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=23376530.

Kniewald, J., et al., Jan-Feb 2000. Disorders of male rat reproductive tract under the influence of atrazine. J. Appl. Toxicol. 20 (1), 61–68. ISSN 0260-437X (Print), 0260-437X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10641017.

Laville, N., et al., Nov 10 2006. Modulation of aromatase activity and mRNA by various selected pesticides in the human choriocarcinoma JEG-3 cell line. Toxicology 228 (1), 98–108. ISSN 0300-483X (Print), 0300-483X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/16996190.

Lee, K.H., et al., Nov 2001. Estrogen regulation of ion transporter messenger RNA levels in mouse efferent ductules are mediated differentially through estrogen receptor (ER) alpha and ER beta. Biol. Reprod. 65 (5), 1534–1541. ISSN 0006-3363 (Print), 0006-3363 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/11673272.

Lee, K.H., et al., Jun 2009. Morphological comparison of the testis and efferent ductules between wild-type and estrogen receptor alpha knockout mice during postnatal development. J. Anat. 214 (6), 916–925. ISSN 1469-7580 (Electronic), 0021-8782 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/19538635.

Lenkowski, J.R., et al., Feb 2008. Perturbation of organogenesis by the herbicide atrazine in the amphibian Xenopus laevis. Environ. Health Perspect. 116 (2),

223–230. ISSN 0091-6765 (Print), 0091-6765 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/18288322.
Lewis, S.R., et al., Feb 2014. Steroidogenic factor 1 promotes aggressive growth of castration-resistant prostate cancer cells by stimulating steroid synthesis and cell proliferation. Endocrinology 155 (2), 358–369. ISSN 1945-7170 (Electronic), 0013-7227 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/24265454.
Liu, X.M., et al., Feb 2006. Cytotoxic effects and apoptosis induction of atrazine in a grass carp (Ctenopharyngodon idellus) cell line. Environ. Toxicol. 21 (1), 80–89. ISSN 1520-4081 (Print), 1520-4081 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/16463256.
Mahendroo, M.S., Mendelson, C.R., Simpson, E.R., Sep 15 1993. Tissue-specific and hormonally controlled alternative promoters regulate aromatase cytochrome P450 gene expression in human adipose tissue. J. Biol. Chem. 268 (26), 19463–19470. ISSN 0021-9258 (Print), 0021-9258 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/7690033.
Martins-Santos, E., et al., Aug 25 2017. Persistent testicular structural and functional alterations after exposure of adult rats to atrazine. Reprod. Toxicol. 73. ISSN 1873-1708 (Electronic), 0890–6238 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/28847621.
Mckinnell, C., et al., Mar-Apr 2001. Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol. J. Androl. 22 (2), 323–338. ISSN 0196-3635 (Print), 0196-3635 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/11229807.
Nakai, M., et al., Nov-Dec 1992. Acute and long-term effects of a single dose of the fungicide carbendazim (methyl 2-benzimidazole carbamate) on the male reproductive system in the rat. J. Androl. 13 (6), 507–518. ISSN 0196-3635 (Print), 0196-3635 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/1293130.
Nakai, M., Moore, B.J., Hess, R.A., Jan 1993. Epithelial reorganization and irregular growth following carbendazim-induced injury of the efferent ductules of the rat testis. Anat. Rec. 235 (1), 51–60. ISSN 0003-276X (Print), 0003-276X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/8417628.
Nanjappa, M.K., et al., Jul 2016. Membrane-localized estrogen receptor 1 is required for normal male reproductive development and function in mice. Endocrinology 157 (7), 2909–2919. ISSN 1945-7170 (Electronic), 0013-7227 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/27145009.
Oliveira, A.G., et al., Dec 2007. 5alpha-Androstane-3beta,17beta-diol (3beta-diol), an estrogenic metabolite of 5alpha-dihydrotestosterone, is a potent modulator of estrogen receptor ERbeta expression in the ventral prostrate of adult rats. Steroids 72 (14), 914–922. ISSN 0039-128X (Print), 0039-128X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/17854852.
Oliveira, C.A., et al., Jun 2005. Aquaporin-1 and -9 are differentially regulated by oestrogen in the efferent ductule epithelium and initial segment of the epididymis. Biol. Cell 97 (6), 385–395. ISSN 0248-4900 (Print), 0248-4900 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/15850448.
Oliveira, C.A., et al., Sep 2001. Infertility and testicular atrophy in the antiestrogen-treated adult male rat. Biol. Reprod. 65 (3), 913–920. ISSN 0006-3363 (Print), 0006-3363 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11514358.
Oliveira, C.A., et al., Oct 10 2003. The antiestrogen ICI 182,780 decreases the expression of estrogen receptor-alpha but has no effect on estrogen receptor-beta and androgen receptor in rat efferent ductules. Reprod. Biol. Endocrinol. 1, 75. ISSN 1477-7827 (Electronic), 1477-7827 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/14613549.
Oliveira, C.A., et al., Jun 2002. ER function in the adult male rat: short- and long-term effects of the antiestrogen ICI 182,780 on the testis and efferent ductules, without changes in testosterone. Endocrinology 143 (6), 2399–2409. ISSN 0013-7227 (Print), 0013-7227 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/12021205.
Oliveira, R.L., et al., Jun 01 2013. Differential expression and seasonal variation on aquaporins 1 and 9 in the male genital system of big fruit-eating bat Artibeus lituratus. Gen. Comp. Endocrinol. 186, 116–125. ISSN 1095-6840 (Electronic), 0016-6480 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/23510858.
Pezzi, V., et al., May 2004. Differential expression of steroidogenic factor-1/adrenal 4 binding protein and liver receptor homolog-1 (LRH-1)/fetoprotein transcription factor in the rat testis: LRH-1 as a potential regulator of testicular aromatase expression. Endocrinology 145 (5), 2186–2196. ISSN 0013-7227 (Print), 0013-7227 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/14736734.
Quignot, N., et al., Feb 2012. A comparison of two human cell lines and two rat gonadal cell primary cultures as in vitro screening tools for aromatase modulation. Toxicol Vitro 26 (1), 107–118. ISSN 1879-3177 (Electronic), 0887-2333 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/22120136.
Rey, F., et al., Jul 2009. Prenatal exposure to pesticides disrupts testicular histoarchitecture and alters testosterone levels in male Caiman latirostris. Gen. Comp. Endocrinol. 162 (3), 286–292. ISSN 1095-6840 (Electronic), 0016-6480 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19364509.
Rivest, P., Devine, P.J., Sanderson, J.T., Nov 15 2010. Evaluation of a bioluminescent mouse model expressing aromatase PII-promoter-controlled luciferase as a tool for the study of endocrine disrupting chemicals. Toxicol. Appl. Pharmacol. 249 (1), 33–40. ISSN 1096-0333 (Electronic), 0041-008X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/20723554.
Sanderson, J.T., et al., Jul 1 2002. Induction and inhibition of aromatase (CYP19) activity by various classes of pesticides in H295R human adrenocortical carcinoma cells. Toxicol. Appl. Pharmacol. 182 (1), 44–54. ISSN 0041-008X (Print), 0041-008X (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/12127262.
Sanderson, J.T., et al., Oct 2001. Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. Environ. Health Perspect. 109 (10), 1027–1031. ISSN 0091-6765 (Print), 0091-6765 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11675267.
Sanderson, J.T., et al., Mar 2000. 2-Chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity? Toxicol. Sci. 54 (1), 121–127. ISSN 1096-6080 (Print), 1096-0929 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10746939.
Sanderson, T.J., 2006. The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals. Toxicol. Sci. 1, 18. Disponível em: https://academic.oup.com/toxsci/article-lookup/doi/10.1093/toxsci/kfl051.
Simpson, E.R., Davis, S.R., Nov 2001. Minireview: aromatase and the regulation of estrogen biosynthesis–some new perspectives. Endocrinology 142 (11), 4589–4594. ISSN 0013-7227 (Print), 0013-7227 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/11606422.
Song, X.Y., et al., Jun 12 2015. Atrazine causes autophagy- and apoptosis-related neurodegenerative effects in dopaminergic neurons in the rat nigrostriatal dopaminergic system. Int. J. Mol. Sci. 16 (6), 13490–13506. ISSN 1422-0067 (Electronic), 1422-0067 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/26075868.
Stanko, J.P., et al., Dec 2010. Effects of prenatal exposure to a low dose atrazine metabolite mixture on pubertal timing and prostate development of male Long-Evans rats. Reprod. Toxicol. 30 (4), 540–549. ISSN 1873-1708 (Electronic), 0890-6238 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/20727709.
Stoker, T.E., et al., Nov 2000. The effect of atrazine on puberty in male wistar rats: an evaluation in the protocol for the assessment of pubertal development and thyroid function. Toxicol. Sci. 58 (1), 50–59. ISSN 1096-6080 (Print), 1096-0929 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/11053540.
Stoker, T.E., Robinette, C.L., Cooper, R.L., Nov 1999. Maternal exposure to atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring. Toxicol. Sci. 52 (1), 68–79. ISSN 1096-6080 (Print), 1096-0929 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10568700.
Suzawa, M., Ingraham, H.A., 2008. The herbicide atrazine activates endocrine gene networks via non-steroidal NR5A nuclear receptors in fish and mammalian cells. PLoS One 3 (5), e2117. ISSN 1932-6203 (Electronic), 1932-6203 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/18461179.
Swan, S.H., Feb 2006. Semen quality in fertile US men in relation to geographical area and pesticide exposure. Int. J. Androl. 29 (1), 62–68 discussion 105-8. ISSN 0105-6263 (Print), 0105-6263 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/16466525.
Thibeault, A.A., et al., Apr 2014. A unique co-culture model for fundamental and applied studies of human fetoplacental steroidogenesis and interference by environmental chemicals. Environ. Health Perspect. 122 (4), 371–377. ISSN 1552-9924 (Electronic), 0091-6765 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/24486430.
Tinfo, N.S., et al., Feb 2011. Understanding the effects of atrazine on steroidogenesis in rat granulosa and H295R adrenal cortical carcinoma cells. Reprod. Toxicol. 31 (2), 184–193. ISSN 1873-1708 (Electronic), 0890-6238 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/21126571.
Victor-Costa, A.B., et al., Jun 2010. Changes in testicular morphology and steroidogenesis in adult rats exposed to Atrazine. Reprod. Toxicol. 29 (3), 323–331. ISSN 1873-1708 (Electronic), 0890-6238 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/20045047.
Zhang, X., et al., 2011. Atrazine-induced apoptosis of splenocytes in BALB/C mice. BMC Med. 9, 117. ISSN 1741-7015 (Electronic), 1741-7015 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/22032520.
Zhou, Q., et al., Nov 20 2001. Estrogen action and male fertility: roles of the sodium/hydrogen exchanger-3 and fluid reabsorption in reproductive tract function. Proc. Natl. Acad. Sci. U. S. A. 98 (24), 14132–14137. ISSN 0027-8424 (Print), 0027-8424 (Linking). Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/11698654.