Periódico Eletrônico "Fórum Ambiental da Alta Paulista"
Electronic Journal "Environmental Forum of Alta Paulista"

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

Reproductive effects of chronic oral exposure to glyphosate under environmentally relevant conditions: an experimental study

Jamile Silveira Tomiazzi Simões
Doutora, UNOESTE, Brasil.
jamile.tomiazzi@gmail.com

Mariana Esperendi Bastianini
Doutoranda, UNOESTE, Brasil.
marianaesperendi@gmail.com

Jaqueline Nascimento Silva
Doutora, UNESP, Brasil.
jaqueline.nascimento@unesp.br

Renata Calciolari Rossi
Professora Doutora, UNOESTE, Brasil.
renata@unoeste.br

Ana Paula Alves Favareto
Professora Doutora, UNOESTE, Brasil.
anafavareto@unoeste.br

Periódico Eletrônico "Fórum Ambiental da Alta Paulista"
Electronic Journal "Environmental Forum of Alta Paulista"

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

ABSTRACT

Glyphosate is among the most widely used herbicides globally, raising concerns about its potential adverse health effects. This study aimed to evaluate the reproductive effects of chronic consumption of feed contaminated with glyphosate herbicide in rats. Rats received feed sprayed with glyphosate solution at different concentrations: 0 (control group - CG), 2069 (low concentration group - LCG), 3463 (medium concentration group - MCG), or 5166 ppm per day (high concentration group - HCG) for 180 days. Glyphosate impaired sperm quality. The percentage of sperm with progressive and non-progressive motility and sperm count in the testis was reduced in the two highest exposure groups (MCG and HCG). There was an increase in pinhead and abnormal head and isolated and coiled tail abnormalities in all three exposed groups, with a significant reduction in the number of normal sperm. The integrity of the sperm plasma membrane was not affected by the herbicide. Chronic oral exposure to glyphosate at different concentrations caused alterations in the morphology of adult rat sperm. These findings are crucial as they suggest that prolonged exposure to glyphosate may have detrimental implications on male fertility, highlighting the need for continuous reviews of herbicide use practices and health risk assessments.

KEYWORDS: Herbicide. Reproduction. Environmental.

1 INTRODUCTION

Pesticides are chemicals widely used by agribusiness to facilitate and increase food production. Although this use plays its role in increasing agricultural production, it can have negative consequences on health and the environment (ARL, 2021). Various substances used in agriculture can present teratogenic, mutagenic, and carcinogenic actions, as well as reproductive alterations (KANG et al., 2008; MESNAGE et al., 2018; LEVINE et al., 2020). Exposure to these substances, especially herbicides, occurs not only among rural workers and farmers who handle these compounds directly (occupational exposure) but also among the general population through the ingestion of contaminated water and food (accidental exposure). This exposure can trigger health problems and impact the environment through the degradation of non-renewable resources, imbalance of fauna and flora, and pollution of soil, air, and water (PARANÁ, 2013; IARC, 2018).

One of the most widely used herbicides worldwide is glyphosate (N-phosphonomethyl glycine). It belongs to the chemical class of broad-spectrum, water-soluble, systemic, non-selective, post-emergence organophosphates, comprising more than 750 formulations (CUNHA, 2011). It is the main active ingredient in Roundup® brand herbicides (CUNHA, 2011; MACHADO, 2016; PIGNATI et al., 2017) and is used in cotton, peanut, rice, coffee, sugarcane, cassava, soybean, and wheat crops, as well as in pastures and pine and eucalyptus forests (CETESB, 2022).

The use of pesticides in plantations, the generation of residues, and their accumulation in environmental compartments necessitate thorough studies to identify their behavior in the environment and their impacts on humans and animals (JARDIM et al., 2009). Although glyphosate is classified as practically non-toxic and non-irritating by the US Environmental Protection Agency (EPA, 1993), studies have demonstrated the toxic effects of exposure to pure glyphosate and glyphosate-based herbicides on health and the environment (VANLAEYS et al., 2018; ZHANG et al., 2019). Glyphosate can act as an endocrine disruptor, leading to delayed puberty onset, significant reductions in spermatogenesis and steroidogenesis in prepubertally exposed rats, even at low doses (5 mg/kg) (ROMANO et al., 2012; DAI et al., 2016; JARRELL et al., 2020). Experimental studies on oral exposure to agrochemicals often use gavage, short

exposure periods, and doses that do not resemble human exposure (ARAB et al., 2018; YAHIA et al., 2019), which can occur through direct contamination (inhalation, dermal, and oral) or indirectly through the ingestion of contaminated food and/or water (PEREIRA et al., 2019).

This study aims to evaluate the effects of chronic oral exposure to glyphosate on the reproductive morphology and physiology of adult male rats at environmentally relevant concentrations and adjusted to the agronomic recommendations for herbicide use. Additionally, it proposes an exposure model where animal feed is sprayed with glyphosate solution, similar to how human exposure occurs through the consumption of contaminated food.

2 OBJECTIVES

This study evaluated the effects on the male reproductive system caused by chronic oral exposure to glyphosate herbicide using an experimental model.

3 METHODOLOGY

3.1 Chemical Agents

For glyphosate exposure, the commercial glyphosate-based formulation (Roundup®), registered under number 09106, containing 480 g/L (360 g/L acid equivalent) of glyphosate isopropylamine salt, was used. The formulation was diluted in 10 mL of 0.9% saline solution (sodium chloride - NaCl solution) at the prescribed concentrations for each experimental group.

3.2 Animal Model Study

Adult male Wistar rats (total n = 40) were obtained from the Central Animal Facility of the University of Western São Paulo (UNOESTE), Presidente Prudente, SP, Brazil. During the experiment, these animals were housed in polypropylene cages (43 cm x 30 cm x 15 cm) containing laboratory-quality wood shavings as substrate. Maintenance conditions involved a controlled environment with temperature maintained at 22 ± 2 °C and a light/dark cycle of 12 hours each. Rat chow (Supralab® Alisul Brazil) and unrestricted access to filtered water were provided. The experimental protocol was submitted and approved by the Ethics Committee on Animal Use of UNOESTE (Protocol No. 5684-CEUA) and strictly followed the animal welfare guidelines established by the Brazilian College of Animal Experimentation (COBEA).

3.3 Experimental Groups and Herbicide Exposure Protocol

Rats were randomly distributed into four experimental groups (n = 10 animals per group):

- Control group (CG): rats consumed daily chow previously sprayed with 0.9% saline solution (vehicle);

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

- Low concentration group (LCG): rats consumed daily chow previously sprayed with glyphosate at a concentration of 371 × 10−3 g of active ingredient per hectare (equivalent to 2069 ppm);
- Medium concentration group (MCG): rats consumed daily chow previously sprayed with glyphosate at a concentration of 619 × 10−3 g of active ingredient per hectare (equivalent to 3463 ppm);
- High concentration group (HCG): rats consumed daily chow previously sprayed with glyphosate at a concentration of 928 × 10−3 g of active ingredient per hectare (equivalent to 5166 ppm).

The different concentrations of glyphosate used consider environmentally relevant concentrations according to the application of the product and its agronomic prescription. The concentrations of glyphosate used in agriculture (in grams of active ingredient per hectare - g.i.a) were adjusted to the dimensions of the exposure boxes according to Parizi et al. (2020).

For the chow exposure, a system composed of two plastic boxes (32 x 24 x 32 cm) connected to an ultrasonic nebulizer (Pulmosonic Star® Brazil) was adopted, following the methodology of Mello et al. (2018). The chow was exposed for 180 days. The daily exposure duration was approximately 15 minutes, ensuring complete nebulization of the solution. The exposure occurred one day before the chow was offered to the animals and was changed every two days. Animals were exposed for a period of 180 days.

3.4 Biological Material Collection

At the end of the exposure period, the rats from each experimental group were anesthetized and euthanized by intraperitoneal administration of sodium thiopental at a dose of 100 mg/kg. The right testis and epididymides were collected for sperm analysis.

3.5 Sperm Motility and Morphology and Plasma Membrane Integrity

Immediately after euthanasia, the left vas deferens was collected to obtain sperm in 10 mL of phosphate-buffered saline at 34ºC. The heated Neubauer counting chamber was loaded with a small aliquot of sperm solution. Sperm motility was assessed by visual estimation (200 sperm per animal in duplicate) under a microscope (Leica DMLS) at 200X magnification. Sperm were classified as immobile, mobile without progression, and mobile with progression according to Perobelli et al. (2012).

The right vas deferens was collected to obtain sperm, which were fixed in 10 mL of saline formalin. Sperm morphology analysis was performed under a microscope (400x magnification) (SEED et al., 1996). Sperm were classified according to Filler (1993).

Sperm viability (plasma membrane integrity) was assessed using the eosin-nigrosin staining test (WHO, 1999). Two hundred sperm were counted under a light microscope (1000x magnification) and classified as unstained (live sperm) and stained red (dead sperm).

3.6 Sperm Counts

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

The right testicles were frozen until decapsulated and weighed, then homogenized according to the method described by Robb et al. (1978) with the following adaptations. The testicular parenchyma was thawed and homogenized in a mixture of NaCl (9g) and Triton X100 (0.5ml). After a tenfold dilution in the mixture containing Triton X100, a small sample was transferred to Neubauer chambers (4 fields per animal), and the homogenization-resistant spermatids (stage 19 of spermiogenesis) were counted. To calculate daily sperm production (DSP), the sperm concentration per testicle was divided by 61, which is the number of days the mature spermatids (stage 19 of spermiogenesis) are present in the seminiferous epithelium.

3. 7 Histological Analysis

The left testicles were collected, cleaned, and immersed in 10% buffered formalin solution. After 24 hours of pre-fixation, the organs were removed from the fixative and sectioned, then returned to the fixative. After 24 hours, the pieces were washed, and the solution was replaced with 70% alcohol, where the pieces remained until processing, which consisted of embedding the material in Paraplast, obtaining 5µm thick sections, and staining with hematoxylin and eosin for histopathological evaluation under a light microscope.

Transverse sections of seminiferous tubules were evaluated for alterations such as the presence of acidophilic cells, multinucleated cells, retained spermatids, degeneration of cell types, cell depletion, epithelial vacuolization, or cell exfoliation into the lumen. Longitudinal sections of the head/body and tail segments of the epididymis were examined for the presence of cribriform hyperplasia in the epithelium, epithelial vacuolization, inflammatory infiltrate in the interstitial tissue, and the quantity and appearance of sperm in the lumen and clear cells in the epithelium (KEMPINAS, KLINEFELTER, 2014). Photomicrographs were obtained using a microscope coupled with an image capture system (Leica Microsystems Switzerland).

3.8 Statistical Analysis

The Kolmogorov-Smirnov test was applied to test normal distributions before statistical analyses. For parameter comparisons, ANOVA with Tukey post hoc test or Kruskall-Wallis non-parametric test with Dunn post hoc test were performed. Differences were considered significant when $p < 0.05$.

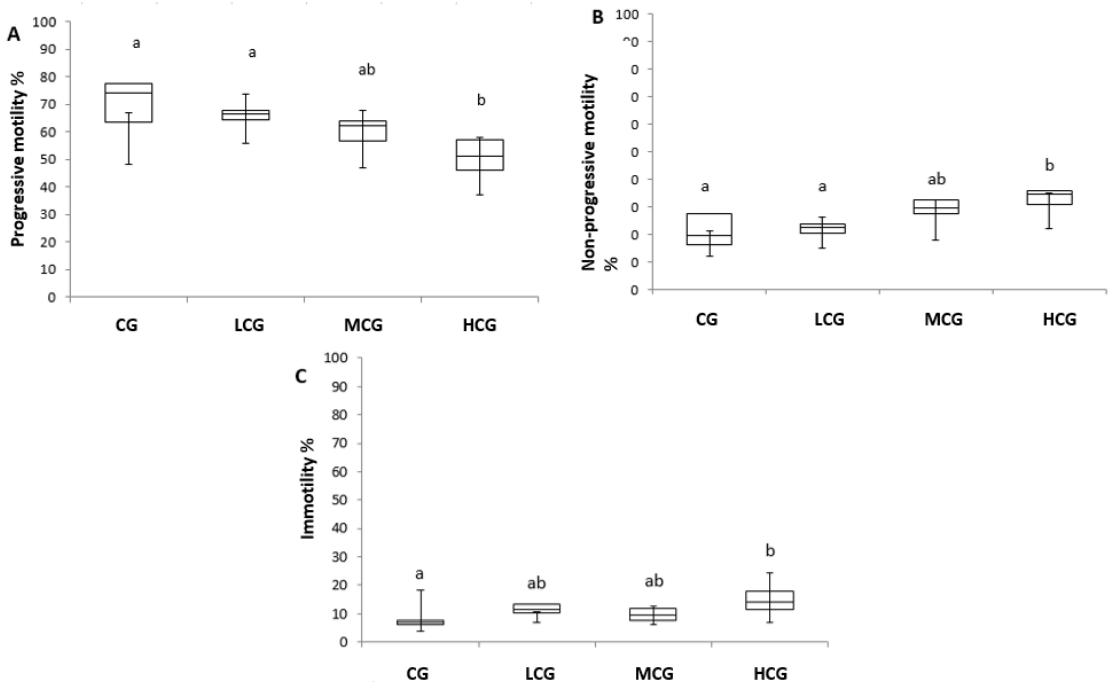
4 RESULTS AND DISCUSSION

In recent years, increasing attention has been given to reproductive toxicity induced by glyphosate and its commercial formulations such as Roundup® (CAI et al., 2017; JARREL et al., 2020). Glyphosate is the most widely used herbicide worldwide, necessitating an understanding of its potential toxic effects and impacts on the male reproductive system due to chronic exposure.

Studies have addressed reduced fertility and sperm parameters in humans (ANIFANDIS et al. 2018), correlating some alterations to pesticide exposure (MANN et al., 2020) and direct

glyphosate use (DALLEGRAVE et al., 2007). The percentage of sperm with progressive motility (Figure 1A) was reduced ($p < 0.05$), with a consequent increase ($p < 0.05$) in the percentage of sperm with non-progressive motility and immotile sperm in HCG compared to CG (Figure 1B and 1C). There was similarity ($p > 0.05$) between LCG, MCG, and CG and between MCG and HCG regarding progressive and non-progressive motility (Figure 1A and 1B). Additionally, LCG and MCG were similar ($p > 0.05$) to CG and HCG in the percentage of immotile sperm (Figure 1C).

Figure 1 – Sperm motility in the four experimental groups: Control (CG) and exposed to glyphosate orally at different concentrations (LCG, MCG, and HCG).



Different letters indicate statistically significant differences between groups ($p < 0.05$).
Source: Prepared by the authors.

This study showed reduced sperm motility characterized by the large number of immotile and non-progressively motile sperm in glyphosate-exposed groups. In vivo studies also show the toxic effect of glyphosate on sperm motility. Guinea pigs exposed to 186, 280, and 560 mg/kg of glyphosate-based herbicides (GBH) (WILLOSATE®) equivalent to 67, 103, and 202 mg/kg of glyphosate respectively, orally for 60 days showed a significant dose-dependent reduction in sperm motility, viability, and concentration (MUTWEDU et al., 2021). Similar results were also previously reported by Owagboriaye et al. (2017) in male albino rats orally exposed for 12 weeks to (0.01, 0.14, and 0.69 mL/kg/day) of Roundup®, corresponding to glyphosate doses of (3.6, 50.4, and 248.4 mg/kg/day), respectively.

Sperm morphology evaluation is also important not only for assessing testicular function but also as an indicator of stress caused by the environment. Sperm defects can reflect alterations during spermatogenesis or sperm maturation and directly interfere with fertilization capacity (NAJAFI 2015).

There was no significant difference ($p > 0.05$) in plasma membrane integrity of sperm exposed to different glyphosate concentrations (Table 1). However, a significant reduction ($p < 0.05$) in the percentage of morphologically normal sperm and an increase in head abnormalities were observed in MCG and HCG compared to CG. LCG maintained similarity ($p > 0.05$) to CG and the other exposed groups. The percentage of tail abnormalities increased ($p > 0.05$) in MCG and HCG, but MCG also maintained similarity ($p > 0.05$) between CG and LCG (Table 1).

The main morphological alterations observed in sperm heads were pinhead and abnormal curvature, while in the tail, isolated and coiled tails were mainly observed.

Mutwedu et al. (2021) also found pinhead and isolated tail sperm head alterations. Sperm quality in glyphosate-exposed groups was impaired, as observed in Nerozzi et al. (2020), which also showed reductions in glyphosate-exposed groups.

Table 1 - Sperm morphology (median (1st quartile - 3rd quartile)) in rats of the four experimental groups: Control (CG) and exposed to glyphosate orally at different concentrations (LCG, MCG, and HCG).

Morphology	CG	LCG	MCG	HCG
Normal (%)	93.50 (89.00-99.00)a	89.75 (85.00-96.00)ab	88.00 (84.00-94.00)b	86.75 (81.00-91.50)b
Anormalidades de cabeça (%)	5.15 (1.00-10.00)a	9.50 (3.00-13.50)ab	10.00 (6.00-14.00)b	11.25 (7.00-16.50)b
Anormalidades de cauda (%)	0.50 (0.00-1.50)a	1.00 (0.50-1.50)a	1.75 (0.00-3.50)ab	2.25 (1.50-6.00)b

Different letters indicate statistically significant differences between groups ($p < 0.05$).
Source: Prepared by the authors.

Sperm counts were altered in this organ. The absolute and relative numbers of sperm in the testis and daily production per testis and per gram of testis were reduced in the three exposed groups compared to CG (Table 2).

Sperm count alterations and reductions in the absolute and relative number of sperm in the testis and daily production per testis and per gram of testis of rats exposed to different glyphosate concentrations have been similarly observed in this study (DAI et al., 2016). Romano et al. (2012) also observed a decrease in the number of sperm, daily sperm production, and an increase in the percentage of abnormal sperm. As with the reduction in sperm production, sperm motility is also directly linked to fertilization, as only mobile sperm reach the oocyte for fertilization.

Reducing sperm motility consequently reduces the reproductive capacity of this sperm (CAI et al., 2017). According to Betancourt et al. (2006), this reduction may occur due to adverse effects on the mitochondrial sheath's respiratory chain, essential for sperm energy supply.

Table 2 - Sperm counts in the testis (mean ± standard deviation) in rats of the four experimental groups: Control (CG) and exposed to glyphosate orally at different concentrations (LCG, MCG, and HCG).

Sperm counts	CG	LCG	MCG	HCG
Sperm number ($x10^6$)	236.15±10.43a	209.08±17.98b	197.49±10.31bc	190.70±8.18c
Sperm number/g ($x10^6$/g)	50.86±2.78a	43.89±4.34b	40.76±3.49b	41.00±3.09b
DSP ($x10^6$/testículo/day)	38.71±1.70a	34.27±2.94b	32.37±1.69bc	31.26±1.34c

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

Relative DSP (x10^6/g/day)	8.33±0.45a	7.19±0.71b	6.68±0.57b	6.72±0.50b

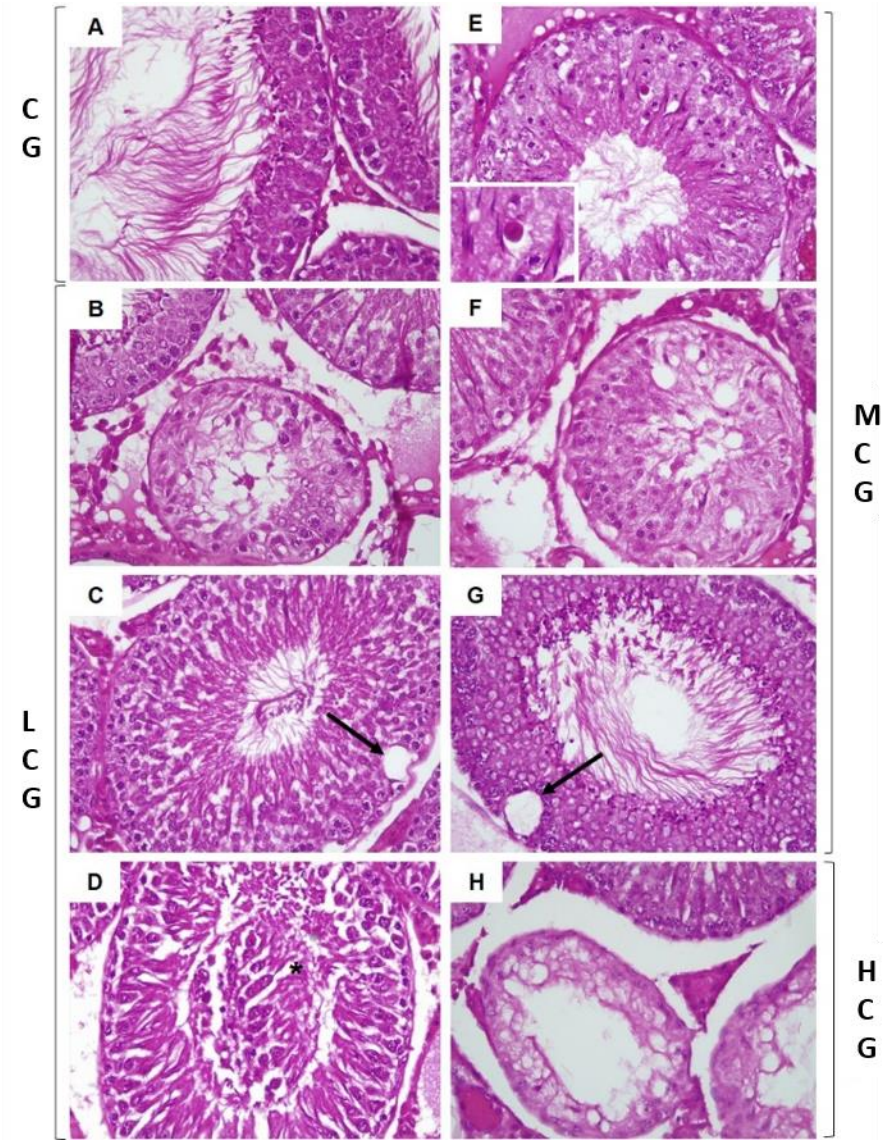
Letras diferentes indicam diferença estatisticamente significativa entre os grupos ($p < 0,05$).
Source: Prepared by the authors.

Regarding histopathological analysis, the testicles of animals in the CG group (Figure 2A) showed normal morphology with organized and preserved germinal epithelium. Testicles of animals exposed to different glyphosate concentrations showed alterations. In LCG, seminiferous tubules with structural disorganization and loss of germ cells were observed (Figure 2B), the presence of exfoliated germ cells in the lumen (Figure 2D), and intraepithelial vacuolization (Figure 2C). In MCG and HCG, the presence of acidophilic germ cells (Figure 2E), seminiferous tubules with intense loss of germ cells and atrophic tubules (Figure 2F and 2H), and intraepithelial vacuolization (Figure 2G) were identified.

The identification of atrophic seminiferous tubules, acidophilic germ cells, and vacuolization of the epithelium and intraepithelial and ductal epithelia is consistent with the study by Vanlaeys et al. (2018), where mice exposed to glyphosate showed similar alterations. These findings indicate a possible reproductive dysfunction and testicular atrophy, conditions that could severely compromise male fertility.

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

Figure 2. Histological analysis of the testis of rats in the four experimental groups: Control (CG) and exposed to glyphosate orally at different concentrations (LCG, MCG, and HCG).



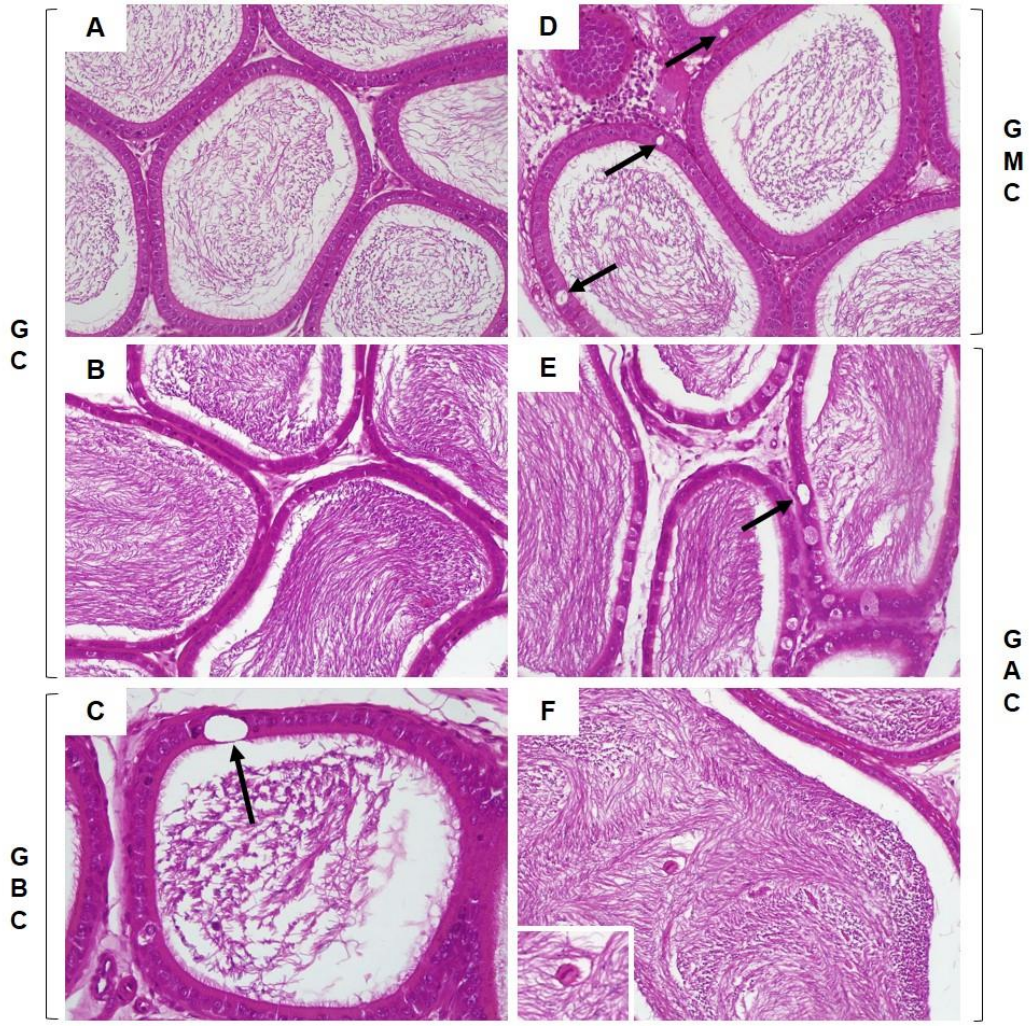
A - Normal structure of seminiferous tubules and interstitial tissue. B, F, H - Loss of structural integrity of seminiferous epithelium with intense loss of germ cells and tubular atrophy. C, G - Presence of seminiferous tubules with intraepithelial vacuolization (arrows). D - Presence of intense exfoliation of germ cells in the lumen of several seminiferous tubules. E – Presence of acidophilic cell. GC = Control group. LCG = Low concentration group. MCG = Medium concentration group. HCG = High concentration group. B, F, H: 200x magnification. A, C-E, G: 400x magnification. Hematoxylin-eosin (HE).

Source: Prepared by the authors.

In the histopathological analysis of the epididymis, CG presented epididymal duct epithelium with normal morphological integrity, interstitium, and lumen with sperm in the head (Figure 3A) and tail (Figure 3B) segments. However, the glyphosate-exposed groups showed focal alterations. Intraepithelial vacuolization of the epididymal duct epithelium was observed in the three herbicide-exposed groups (Figure 3C, 3D, and 3E), and exfoliated immature germ cells in the lumen along with sperm (Figure 3F).

ISSN 1980-0827 – Volume 20, Number 2, Year 2024

Figure 3. Histological analysis of the epididymis of rats in the four experimental groups: Control (CG) and exposed to glyphosate orally at different concentrations (LCG, MCG, and HCG).



A and B - Morphological integrity of the epididymal duct epithelium, interstitium, and lumen with sperm in the head (A) and tail (B) segments. C – E Presence of intraepithelial vacuolization (arrow). E - Epithelium of the cauda epididymis region with a large number of clear cells. A, B, D, E: 200x magnification. C and F: 400x magnification. Hematoxylin-eosin (HE).

Source: Prepared by the authors.

The presence of acidophilic germ cells and intense loss of germ cells in high exposure groups also suggests a severe toxic response to the herbicide, consistent with previous studies (DAI et al., 2016; VANLAEYS et al., 2018), which report the adverse effects of environmental contaminants, including glyphosate, on the morphology and function of reproductive organs.

These results emphasize the need to consider the biological impacts of glyphosate not only at isolated cellular or molecular levels but as an indicator of broader pathophysiological changes affecting complex organ systems. Therefore, continued studies focused on the reproductive toxicity of glyphosate are crucial to fully elucidate the risks associated with its use, supporting the formulation of safer herbicide management and exposure policies.

5 CONCLUSION

The results revealed significant alterations in sperm quality, especially in groups subjected to medium and high concentrations of the herbicide, demonstrating a reduction in motility and normal sperm morphology. The plasma membrane integrity of sperm, on the other hand, showed no significant differences, suggesting that glyphosate's effects are more pronounced on functionality rather than cell viability.

Histological analyses of testicles and epididymis confirmed the presence of morphological and structural alterations in germ cells, reinforcing findings on glyphosate's reproductive toxicity. These results are consistent with previous studies documenting the reproductive toxicity of glyphosate-based herbicides and raise serious concerns about the continued and widespread use of this compound, particularly regarding current safety and exposure recommendations.

Therefore, this study contributes to understanding the biological effects of glyphosate on the male reproductive system and highlights the need for re-evaluation of regulatory practices and herbicide use, considering potential risks to human and environmental health. Future investigations should focus on exploring the underlying mechanisms of the observed effects and evaluating exposures in conditions closer to human scenarios to better contextualize the risks associated with glyphosate.

6 ACKNOWLEDGEMENTS

This study was partially funded by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financial Code 001 and the University of Western São Paulo (UNOESTE).

7 REFERENCES

ANIFANDIS, G. et al. The effect of glyphosate on human sperm motility and sperm DNA fragmentation. **International journal of environmental research and public health**, v. 15, n. 6, p. 1117, 2018. Disponível em: <https://www.mdpi.com/1660-4601/15/6/1117> doi: 10.3390/ijerph15061117.

ARAB, S. A. et al. Evaluation of oxidative stress indices after exposure to malathion and protective effects of ascorbic acid in ovarian tissue of adult female rats. **Electronic physician**, v. 10, n. 5, p. 6789, 2018.

ARL, R. **Toxicidade reprodutiva do glifosato e herbicidas à base de glifosato**: uma abordagem bibliográfica. Trabalho de Conclusão do Curso (Graduação em Farmácia). Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Florianópolis. 2021.

BETANCOURT, M.; RESÉNDIZ, A. Effect of two insecticides and two herbicides on the porcine sperm motility patterns using computer-assisted semen analysis (CASA) in vitro. **Reproductive Toxicology**, v. 22, n. 3, p. 508-512, 2006.

CAI, W. et al. Effects of glyphosate exposure on sperm concentration in rodents: A systematic review and meta-analysis. **Environmental Toxicology and Pharmacology**, v. 55, p. 148-155, 2017. Disponível em: <https://www.sciencedirect.com/science/article/pii/S1382668917302041> doi: 10.1016/j.etap.2017.07.015.

COMPANHIA AMBIENTAL DO ESTADO DE SÃO PAULO – CETESB. **Ficha de Informação Toxicológica, Glifosato**. Divisão de Toxicologia Humana e Saúde Ambiental, 2022. Disponível em: <https://cetesb.sp.gov.br/laboratorios/wpcontent/uploads/sites/24/2022/02/Glifosato.pdf>

CUNHA, F. L. **Agrotóxicos no Brasil: um guia para ação em defesa da vida**. Rio de Janeiro: AS-PTA-Assessoria e Serviços a Projetos em Agricultura Alternativa, v. 1, p. 190, 2011.

DAI, P. et al. Effect of glyphosate on reproductive organs in male rat. **Acta Histochemica**, v. 118, n. 5, p. 519-526, 2016.

DALLEGRAVE, E. et al. Pre-and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. **Archives of toxicology**, v. 81, n. 9, p. 665-673, 2007. Disponível em: <https://link.springer.com/article/10.1007/s00204-006-0170-5>. doi: 10.1007/s00204-006-0170-5.

FILLER, R. Methods for evaluation of rat epididymal sperm morphology. **Methods in Toxicology**, v. 3, p. 334-343, 1993. Disponível em: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6033125/pdf/epj-10-6789.pdf>. doi: 10.19082/6789.

IARC, Working Group on the Evaluation of Carcinogenic Risks to Humans. **Some Organophosphate Insecticides and Herbicides**. v. 112, 2017.

JARDIM, I. C. S. F. et al. Resíduos de agrotóxicos em alimentos: uma preocupação ambiental global - Um enfoque às maçãs. **Química Nova,** n. 32, v. 4, 2009. doi: 10.1590/S0100-40422009000400031

JARREL, Z. R.; AHAMMAD, M. U.; BENSON, A. P. Glyphosate-based herbicide formulations and reproductive toxicity in animals. **Veterinary and animal science**, v. 10, 100126, 2020.

KANG, D. et al. Cancer incidence among pesticide applicators exposed to trifluralin in the Agricultural Health Study. **Environ Res**, v. 107, n. 2, p. 271-276, 2008. doi: 10.1016/j.envres.2008.01.010.

KEMPINAS, W.D.G.; KLINEFELTER, G.R. Interpreting histopathology in the epididymis. **Spermatogenesis**, v. 4, n. 2, p. 1-12, 2014. doi: 10.4161/21565562.2014.979114.

LEVINE, S. L.; WEBB, E. G.; SALTMIRAS, D. A. Review and analysis of the potential for glyphosate to interact with the estrogen, androgen and thyroid pathways. **Pesticides Management Science**, v. 76, n. 9, p. 2886-2906, 2020.

MACHADO, M. O. **Glifosato**: a emergência de uma controvérsia científica global. 315f. Tese (Doutorado em Ciências Humanas). Universidade Federal de Santa Catarina, Florianópolis. 2016. Disponível em: <https://repositorio. ufsc. br/handle/123456789/169662>.

MANN, U. et al. Reasons for worldwide decline in male fertility. **Curr Opin Urol,** v. 30, n. 3, p. 296-301, 2020. doi: 10.1097/MOU.0000000000000745.

MELLO, F. A. et al. Evaluation of the nasal cavity mice submitted to the inhalation exposure to the herbicide 2, 4-dichlorophenoxyacetic acid. **Medicina (Ribeirão Preto, Online)**, v. 51, n. 4, p. 247-253, 2018. doi: 10.11606/issn.2176-7262.v51i4p247-253.

MESNAGE, R.; BERNAY, B.; SÉRALINI, G. E. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. **Toxicology**, v. 313, n. 2-3, p. 122-128, 2018.

MUTWEDU, V. B. et al. Growth performance and reproductive function impairment of glyphosate-based herbicide in male guinea pig (Cavia porcellus). **Veterinary Medicine and Science**, v. 7, n. 3, p. 1047-1055, 2021.

NAJAFI, T.F. et al. Air pollution and quality of sperm: a meta-analysis. **Iranian Red Crescent Medical Journal**, v. 15, n.4, e26930, 2015. doi: 10.5812/ircmj.17(4)2015.26930.

NEROZZI, C. et al. Effects of Roundup and its main component, glyphosate, upon mammalian sperm function and survival. **Scientific reports**, v. 10, n. 1, p. 1-9, 2020.

OWAGBORIAYE, F. O. et al. Reproductive toxicity of Roundup herbicide exposure in male albino rat. **Experimental and Toxicologic Pathology**, v. 69, n. 7, p. 461-468, 2017.

PARANÁ. Secretaria de Estado da Saúde do Paraná. Superintendência de Vigilância em Saúde. Centro Estadual de Saúde do Trabalhador. **Protocolo de avaliação das intoxicações crônicas por agrotóxicos**, p. 66, 2013.

PARIZI, J.L.S. et al. Oral mucosa changes associated with chronic oral and inhalation exposure to 2,4-dichlorophenoxiacetic acid (2,4-D) in Wistar rats. **Toxicol Res (Camb)**, v. 9, n. 6, p. 746-757, 2020. doi: 10.1093/toxres/tfaa085.

PEREIRA, V. R. et al. Sperm quality of rats exposed to difenoconazole using classical parameters and surface-enhanced Raman scattering: classification performance by machine learning methods. **Environ Sci Pollut Res Int**, v. 26, n. 34, p. 35253-35265, 2019. doi: 10.1007/s11356-019-06407-0.

PEROBELLI, J. E. et al. Impairment on sperm quality and fertility of adult rats after antiandrogen exposure during prepuberty. **Reproductive toxicology**, v. 33, n. 3, p. 308-315, 2012. doi: 10.1016/j.reprotox.202011.12.011.

PEROBELLI, J.E. et al. Impairment on sperm quality and fertility of adult rats after antiandrogen exposure during prepuberty. **Reprod Toxicol,** v. 33, p. 308–315, 2012. doi: 10.1016/j.reprotox.202011.12.011.

PIGNATI, W. A. et al. Distribuição espacial do uso de agrotóxicos no Brasil: uma ferramenta para a Vigilância em Saúde. **Ciência & Saúde Coletiva**, v. 22, 3281-3293, 2017.

ROBB, G. W.; AMANN, R. P.; KILLIAN, G. J. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. **Reproduction**, v. 54, n. 1, p. 103-107, 1978. doi: 10.1530/jrf.0.0540103.

ROMANO, M. A. et al. Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. **Archives of toxicology**, v. 86, n. 4, p. 663-673, 2012.

SEED, J. et al. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. ILSI risk science institute expert working group on sperm evaluation. **Reprod Toxicol**, v. 10, n. 3, p. 237–244, 1996. doi: 10.1016/0890-6238(96)00028-7.

US EPA. **Reregistration eligibility decision (RED)**: glyphosate. Office of Prevention, Pesticides And Toxic Substances, setembro 1993. Disponível em: <http://archive.epa.gov/pesticides/reregistration/web/pdf/glyphosate.pdf>

VANLAEYS, A.; DUBUISSON, F.; SERALINI, G. E.; TRAVERT, C. Formulants of glyphosate-based herbicides have more deleterious impact than glyphosate on TM4 Sertoli cells. **Toxicology in Vitro**, v. 52, p. 14-22, 2018.

YAHIA, D.; EL-AMIR, Y. O.; RUSHDI, M. Mancozeb fungicide-induced genotoxic effects, metabolic alterations, and histological changes in the colon and liver of Sprague Dawley rats. **Toxicology and industrial health**, v. 35, n. 4, p. 265-276, 2019. Disponível em: <https://journals.sagepub.com/doi/abs/10.1177/0748233719834150>. doi: 10.1177/0748233719834150.

ZHANG, J. W.; XU, D. Q.; FENG, X. Z. The toxic effects and possible mechanisms of glyphosate on mouse oocytes. **Chemosphere**, v. 237, 124435, 2019.