Revista AgroTecnologia, Anápolis v. 16, n.1, p.01-11, 2025
ISSN eletrônico: 2179-5959 / ISSN impresso: 2179-5940
Universidade Estadual de Goiás

EFFECTS OF EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE ON MALE MICE REPRODUCTION

EFEITOS DA EXPOSIÇÃO A UM HERBICIDA À BASE DE GLIFOSATO NA REPRODUÇÃO MASCULINA DE CAMUNDONGOS

Thamyres Cunha RODRIGUES*[1] •Renata Karine de CARVALHO*[1] •Walter DIAS JÚNIOR*[2] •Caio César BARBOSA*[1] •Mônica Levy ANDERSEN*[3] •Renata MAZARO-COSTA*✉[1]

Abstract

Glyphosate is classified as a post-emergent herbicide with a broad spectrum of activity and is the most widely sold pesticide in Brazil and globally. Glyphosate is also recognized as an endocrine disruptor capable of inducing reproductive disorders in animals. Therefore, this study aimed to evaluate the effects of a glyphosate-based herbicide (Gliz 480 SL) on the reproductive function of male mice during different exposure periods. The following parameters were analyzed: testicular germinal epithelium, daily sperm production, and sperm transit time through the epididymis. A total of 48 adult Swiss mice were divided into three groups exposed to glyphosate at a daily dose of 0.35 mg/kg for 15, 30, or 60 days, and three control groups that received only water (vehicle). A reduction in the relative mass of the caput/corpus of the epididymis, accompanied by a shorter sperm transit time, was observed after 15 days of exposure, resulting in increased sperm accumulation in the cauda epididymis. Stages V–VI of spermatogenesis showed reductions during 15 and 60 days of exposure. Based on the applied protocol, the glyphosate formulation Gliz 480 SL can alter reproductive parameters, particularly during short exposure periods.

Keywords: Spermatogenesis. Seminiferous epithelium. Epididymis.

Resumo

O glifosato é o pesticida mais comercializado no Brasil e no mundo, sendo classificado como herbicida de uso pós-emergente e com amplo espectro de ação. O glifosato é conhecido como disruptor endócrino, podendo causar distúrbios reprodutivos em animais. Dessa forma, o objetivo deste estudo foi avaliar os efeitos de um herbicida à base de glifosato (Gliz 480 SL) em parâmetros do sistema reprodutor masculino de camundongos em diferentes períodos de exposição. Tais parâmetros incluíram a análise do epitélio germinativo do testículo, cálculo da produção diária de espermatozoides e a avaliação do tempo de trânsito espermático pelo epidídimo. Para isso, foram utilizados 48 camundongos *Swiss* adultos distribuídos em 3 grupos que receberam uma solução de glifosato na dose diária de 0,35 mg/kg, durante os períodos de 15, 30 e 60 dias e, 3 grupos Controle expostos apenas a água (veículo). Durante o período de 15 dias de exposição houve redução na massa relativa da cabeça/corpo do epidídimo, acompanhada pela diminuição do tempo de trânsito dos espermatozoides pela unidade cabeça/corpo do epidídimo, resultando no aumento de espermatozoides na cauda epididimária. Os estágios V–VI da espermatogênese foram reduzidos durante os períodos de 15 e 60 dias de exposição. Infere-se, pelo protocolo utilizado, que a formulação de glifosato Gliz 480 SL pode alterar parâmetros reprodutivos, principalmente durante períodos curtos de exposição.

Palavras-Chaves: Espermatogênese. Epitélio seminífero. Epidídimo.

✉ *Renata Mazaro-Costa, mazaro@ufg.br*

Endereço: [1] Instituto de Ciências Biológicas, Universidade Federal de Goiás, Samambaia Campus, Goiânia, Goiás, Brasil
[2] Universidade Estadual de Goiás
[3] Universidade Federal de São Paulo

ORCID:

Carvalho: https://orcid.org/0000-0001-8263-3896,
Dias Júnior: https://orcid.org/0000-0003-3019-7089,
Barbosa: https://orcid.org/0000-0002-8072-1793,
Andersen: https://orcid.org/0000-0002-1894-6748,
Mazaro-Costa: https://orcid.org/0000-0002-0198-2910.

Introduction

The modernization of agriculture in Brazil during the mid-20th century was closely linked to the development and production of pesticides, which primarily aimed to support population growth by increasing food production (KLEIN; LUNA, 2023). Currently, a major challenge in the agricultural sector involves producing pesticides that are effective, biologically safe, and environmentally sustainable. Many pesticides are employed in agriculture, especially herbicides. Since the 1940s, numerous herbicides with distinct modes of action have been developed and sold (UMETSU; SHIRAI, 2020).

The most widely sold herbicides worldwide are based on glyphosate (N-[phosphonomethyl]-glycine), a non-selective compound used to control weeds competing with crops (DILL et al., 2010). Compared with most herbicides, glyphosate ($C_3H_8NO_5P$) has a relatively short half-life in soil and water (1 to 280 days), primarily due to microbial degradation. Its main metabolite, aminomethylphosphonic acid, exhibits slightly greater mobility and persistence in soil, with a half-life ranging from 23 to 958 days. Thus, this metabolite is more detected in surface and groundwater (COSTAS-FERREIRA et al., 2022).

Glyphosate-based herbicides (GBH) are commonly low toxic for mammals since they inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, specific to plants and certain microorganisms (DILL et al., 2010). However, the toxicity of glyphosate and related formulations remains controversial, particularly concerning the potential carcinogenic effects (RANA et al., 2023). Additionally, glyphosate has been identified as an endocrine disruptor (PHUSATE, 2022) associated with neurological disorders in humans (COSTAS-FERREIRA et al., 2022).

Recently, the National Health Surveillance Agency (ANVISA) revised the use of glyphosate in Brazil and decided to maintain its use with restrictions. Based on reports from international regulatory agencies, the ANVISA increased the acceptable daily intake (ADI) of glyphosate from 0.042 mg/kg to 0.5 mg/kg (DE ARAUJO et al., 2023). The justification included insufficient scientific evidence to demonstrate greater risks to human health beyond those identified in laboratory animal tests. Consequently, further research is crucial to assess the risks associated with glyphosate use.

In this context, this study aimed to assess the effects of different exposure periods to GBH on the male reproductive function of mice using a dose equivalent to the ADI established by ANVISA before the 2023 update. The testicular germinal epithelium, daily sperm production, and sperm transit time through the epididymis were assessed. These analyses are relevant in reproductive toxicology and considered sensitive markers of male reproductive function. The analysis of germ cells progressing through distinct developmental stages within the seminiferous tubules enables the assessment of integrity and efficiency of sperm production, an important indicator of testicular function (HESS; DE FRANCA, 2009). Sperm transit time through the epididymis also provides critical information regarding sperm maturation, a key process for male fertility (CORNWALL, 2009). To date, this study is the first to investigate the effects of the commercial glyphosate formulation Gliz 480 SL on the reproductive function of male Swiss mice across different exposure periods.

Materials and Methods

The chemical compound

The commercial formulation Gliz 480 SL, containing 480 g/L (48% w/v) of glyphosate isopropylamine salt and 356 g/L (35.6% w/v) of glyphosate acid equivalent, was provided by a local farmer. The formulation was diluted in water before administration. The molecular weight of glyphosate (CAS 1071-83-6) is 169.05 g/mol.

Mice and experimental groups

Sexually mature Swiss mice (50 days old, 40 g of weight) were obtained from the Center for Production and Science in Biomodels at the Federal University of Goiás (UFG) and maintained under controlled conditions (23 °C and 12/12 h light/dark cycle) at the Laboratory of Toxicological Physiology and Biochemistry, State University of Goiás – Ceres Campus (UEG - CERES/GO), with food and water *ad libitum*.

Mice were randomly assigned to six experimental groups (n = 8 per group) and treated by oral gavage with 0.35 mg/kg of GBH diluted in water or water alone (control groups) for 15, 30, or 60 consecutive days. The dosage used was based on the ADI for humans in Brazil (0.042 mg/kg) and adjusted according to body surface area (Reagan-

Shaw et al., 2000). All procedures were approved by the ethics committee on animal use under protocol number 047/2016-CEUA/UFG.

Body mass, food, and water intake

Body mass and food and water intake were recorded three times per week throughout the treatment period until euthanasia.

Organ and tissue mass

Following euthanasia by decapitation, the testes, epididymides (caput/corpus and cauda segments), prostate, seminal vesicle, retroperitoneal and epididymal adipose tissue, soleus and gastrocnemius muscles, and adrenal glands were weighed to determine the absolute and relative mass for each animal.

Assessment of blood glucose

Blood samples for glucose assessment were collected during decapitation using the enzymatic glucose oxidation method, in which glucose oxidase produces antipyrilquinonimine. The resulting compound presents a reddish coloration, with intensity measured by spectrophotometry at a maximum absorption wavelength of 510 nm. Glucose concentration (in mg/dL) is directly proportional to the color intensity produced.

Sperm count, daily sperm production, and sperm transit time through the epididymis

The right testes and epididymides, previously frozen at -20°C, were thawed for the estimation of mature spermatid numbers, daily sperm production (DSP), and sperm transit time through the epididymis, according to Souza et al. (2019) with minor adaptations. Briefly, the testis was decapsulated and homogenized in a solution containing NaCl (0.9%) and Triton X-100 (0.05%), followed by ultrasonic bath treatment. After dilution, an aliquot was transferred to Neubauer chambers for counting homogenization-resistant spermatids. DSP was calculated by dividing the total number of spermatids by 4.84, which corresponded to the number of days mature spermatids remain in the seminiferous epithelium of mice. Caput/corpus and cauda segments of the epididymis were homogenized, and spermatozoa were counted. Sperm transit time through the epididymis was estimated by dividing the number of spermatozoa in each epididymal region by DSP.

Germinal epithelium dynamics

The left testes were used in histological analysis to assess the spermatogenesis and Sertoli cell counts. For this, tissues (n = 6 per group) were sectioned, mounted on microscope slides, and stained with hematoxylin and eosin. Spermatogenesis was analyzed by counting 105 cross-sections of seminiferous tubules per mouse. Within these sections, spermatogenic stages were classified according to the following criteria adapted from Hess and de Franca (2009) and Souza et al. (2019): stages I–IV (type A and intermediate spermatogonia, pachytene spermatocytes, and two generations of spermatids), stages V–VI (types A and B spermatogonia, pachytene spermatocytes, and two generations of spermatids), stages VII–VIII (type A spermatogonia, preleptotene, and pachytene spermatocytes, and two generations of spermatids), stage IX (type A spermatogonia, leptotene and pachytene spermatocytes, and one generation of spermatids), stages X–XI (type A spermatogonia, leptotene and zygotene spermatocytes, and one generation of spermatids), and stage XII (meiosis I and II, with zygotene spermatocytes and one generation of spermatids).

Statistical analysis

Two-way analysis of variance (two-way ANOVA) followed by Tukey's post hoc test was performed to assess differences between groups (Control and GBH) and treatment periods. Statistical significance was set at $p < 0.05$.

Results and Discussion

The impacts of the exposure to GBH were primarily observed after 15 days, with significant alterations in spermatogenesis stages and epididymal reproductive parameters.

Somatic parameters

As shown in Table 1, exposure to GBH at a dose of 0.35 mg/kg for 15, 30, and 60 days did not produce evident signs of toxicity regarding food ($F_{(2.98)} = 1.242$; $p = 0.293$) or water intake ($F_{(2.98)} = 0.922$; $p = 0.401$). This result was consistent with body mass, which was also not significantly affected ($F_{(2.53)} = 0.876$; $p = 0.422$).

Table 1 - Somatic parameters of adult Swiss mice (n = 8 per group) treated for 15, 30, and 60 days with 0.35 mg/kg of GBH or water (control groups).

Parameters	Experimental groups					
	Control			GBH		
	15 days	30 days	60 days	15 days	30 days	60 days
Body mass gain (g)	2.0±5.0	1.5±2.1	1.2±4.0	0.9±1.3	1.1±4.3	2.3±2.5
Food intake (g)	73.2±31.4	64.7±21.0	70.3±15.1	87.4±39.7	73.9±21.9	67.4±22.3
Water intake (mL)	90.4±37.1	93.7±13.6	99.8±30.6	100.0±40.2	91.2±23.7	87.6±31.7

Data analyzed using two-way ANOVA followed by Tukey's post hoc test and expressed as mean ± standard deviation ($p < 0.05$). GBH: Glyphosate-based herbicide.

Similarly, mice exposed to higher doses of GBH (60 to 540 mg/kg) for 30 days or longer showed no alterations in body mass (JIANG et al., 2018). Supporting data on water and food intake and body mass evaluated in this study, glucose concentration (Figure 1) was not significantly different between GBH groups and controls ($F_{(2.24)} = 1.260$; $p = 0.302$).

Glyphosate has been shown to act as an endocrine disruptor capable of reducing serum concentrations of reproductive hormones, such as testosterone (PHUSATE, 2022), which may consequently affect the mass of male reproductive organs (DAI et al., 2016). Among the reproductive organs analyzed in this study (Table 2), only the caput/corpus segment of the epididymis from mice exposed to GBH for 15 days showed a reduction of approximately 35% in relative mass compared with controls ($F_{(2.42)} = 5.637$; $p = 0.007$). The absolute and relative masses of the testes, cauda epididymis, prostate, and seminal vesicle were similar to their respective control groups (15, 30, and 60 days).

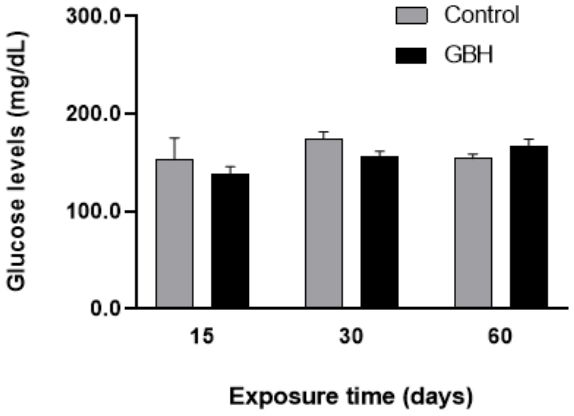

Figure 1. Blood glucose concentration (mg/dL) of adult Swiss mice (n = 5 per group) treated for 15, 30, and 60 days with 0.35 mg/kg of GBH or water (control groups). Data analyzed using two-way ANOVA followed by Tukey's post hoc test and expressed as mean ± standard deviation ($p < 0.05$). GBH: glyphosate-based herbicide.

Table 2. Absolute and relative masses of reproductive organs from male Swiss mice (n = 8 per group) treated for 15, 30, and 60 days with 0.35 mg/kg of GBH or water (control groups).

Parameters	Experimental groups					
	Control			GBH		
	15 days	30 days	60 days	15 days	30 days	60 days
Absolute (g)						
Final body mass	50.0±3.7	44.9±4.5	49.6±3.4	56.0±4.8	48.3±5.5	51.0±4.9
Testis	0.123±0.016	0.117±0.014	0.126±0.031	0.117±0.040	0.127±0.024	0.125±0.016
Caput/corpus epididymis	0.034±0.007	0.025±0.004	0.030±0.005	0.026±0.007	0.028±0.004	0.032±0.006
Cauda epididymis	0.016±0.003	0.012±0.004	0.019±0.003	0.018±0.013	0.013±0.004	0.019±0.002
Prostate	0.185±0.053	0.146±0.050	0.198±0.050	0.209±0.104	0.201±0.053	0.160±0.051
Full seminal vesicle	0.133±0.041	0.085±0.028	0.136±0.042	0.125±0.016	0.105±0.034	0.137±0.037
Relative (mg.100g^{-1})						
Testis	0.246±0.035	0.259±0.041	0.259±0.070	0.218±0.072	0.262±0.041	0.246±0.033
Caput/corpus epididymis	0.069±0.015	0.056±0.012	0.061±0.008	0.046±0.012*	0.058±0.006	0.063±0.016
Cauda epididymis	0.033±0.007	0.027±0.008	0.039±0.007	0.033±0.024	0.026±0.008	0.037±0.007
Prostate	0.368±0.089	0.321±0.095	0.399±0.099	0.368±0.165	0.416±0.093	0.311±0.079
Full seminal vesicle	0.264±0.071	0.187±0.051	0.275±0.089	0.224±0.031	0.216±0.057	0.267±0.060

Data analyzed using two-way ANOVA followed by Tukey's post hoc test and expressed as mean ± standard deviation. *Significant differences between GBH-treated and controls ($p < 0.05$). GBH: Glyphosate-based herbicide.

The epididymis is an androgen-dependent organ that can indicate variations in testosterone concentration (CORNWALL, 2009). Thus, the reduction in epididymal mass observed in the group exposed to GBH for 15 days may reflect decreased testosterone levels. GBH may interfere with steroidogenesis either indirectly by inhibiting hypothalamic-pituitary hormones or directly by downregulating proteins or genes encoding key steroidogenic enzymes (PANDEY; RUDRAIAH, 2015; ZHAO et al., 2021). Since reproductive hormones were not assessed in this study, future investigations are needed to confirm these hypotheses.

Alterations in the epididymis provide relevant information regarding reproductive toxicity since the reduction in relative epididymal mass was not accompanied by changes in body mass. Notably, the relative epididymal mass of the caput/corpus segment returned to control levels with increased exposure time. Mice exposed to GBH for 30 and 60 days likely experienced a longer adaptation period to the herbicide, preventing reductions in epididymal mass. This pattern of recovery aligns with a hormetic response, in which exposure to low doses of a chemical agent triggers an initial disruption of homeostasis followed by an adaptive compensatory response (MATTSON, 2007).

Toxicological evaluations of the soleus and gastrocnemius muscles, epididymal and retroperitoneal adipose tissues, and adrenal glands were also conducted, but no significant alterations indicative of toxicity were observed (Table 3).

Table 3. Absolute and relative masses of non-reproductive organs and tissues from adult Swiss mice (n = 8 per group) treated for 15, 30, and 60 days with 0.35 mg/kg of GBH or water (control groups).

Parameters	Experimental groups					
	Control			GBH		
	15 days	30 days	60 days	15 days	30 days	60 days
Absolute (g)						
Final body mass	50.0±3.7	44.9±4.5	49.6±3.4	56.0±4.8	48.3±5.5	51.0±4.9
Soleus muscle	0.013±0.002	0.010±0.002	0.013±0.007	0.009±0.005	0.011±0.004	0.012±0.004
Gastrocnemius muscle	0.223±0.027	0.225±0.020	0.232±0.063	0.214±0.054	0.207±0.034	0.216±0.027
EA tissue	0.461±0.252	0.512±0.240	0.507±0.194	0.618±0.142	0.630±0.304	0.550±0.123
RA tissue	0.241±0.185	0.217±0.134	0.282±0.133	0.335±0.144	0.347±0.198	0.242±0.112
Right adrenal gland	0.006±0.005	0.003±0.001	0.005±0.003	0.004±0.002	0.003±0.001	0.004±0.002
Relative (mg.100g^{-1})						
Soleus muscle	0.026±0.004	0.021±0.005	0.026±0.016	0.016±0.010	0.022±0.008	0.024±0.008
Gastrocnemius muscle	0.447±0.051	0.505±0.062	0.466±0.110	0.379±0.089	0.429±0.049	0.427±0.071
EA tissue	0.914±0.466	1.118±0.487	1.013±0.337	1.105±0.238	1.261±0.511	1.074±0.184
RA tissue	0.470±0.327	0.465±0.263	0.562±0.234	0.587±0.222	0.689±0.329	0.468±0.196
Right adrenal gland	0.012±0.011	0.007±0.002	0.010±0.007	0.008±0.005	0.007±0.003	0.007±0.004

Data analyzed using two-way ANOVA followed by Tukey's post hoc test and expressed as mean ± standard deviation ($p < 0.05$). EA: Epididymal adipose tissue; GBH: Glyphosate-based herbicide; RA: Retroperitoneal adipose tissue.

In rodents, the visceral adipose tissue primarily consists of epididymal and retroperitoneal adipose tissues (MAUER et al., 2001). Accumulation of these tissues may increase body mass and lead to the development of metabolic disorders, such as insulin resistance, which may result in hyperglycemia (GABRIELY et al., 2002). The absolute and relative masses of epididymal and retroperitoneal adipose tissues observed in mice exposed to GBH corresponded to body mass gain and blood glucose concentration, with no significant alterations following treatment.

Conversely, skeletal striated muscles are considered indicators of malnutrition. During muscle development, nutritional deficiency may result in irreversible reductions in the number of muscle fibers. In cases of malnutrition in stages before development, muscle fiber quantity remains unaffected while fiber thickness decreases, influencing overall muscle mass (Parada-Simão et al., 2011). No significant differences were observed in soleus or gastrocnemius muscle mass, consistent with food intake between the control and GBH groups.

The assessment of adrenal gland mass contributes to an indirect measure of steroidogenic function under GBH treatment. Corticosteroids, including corticosterone, are considered reliable physiological indicators of stress in rodents, similar to immobilization and exposure to toxicants. Alterations in adrenal mass may reflect impairments in the synthesis and release of steroid hormones, including corticosteroids and sex steroids, essential for reproductive maintenance (PANDEY; RUDRAIAH, 2015). However, this study showed no alterations in adrenal mass.

Spermatogenesis

The evaluation of germinal epithelium stages, grouped in this study as stages I–IV, V–VI, VII–VIII, IX, X–XI, and XII, is presented in Table 4. Only the stages V–VI exhibited significant differences between groups. The frequency of stages V–VI (proliferative stages of spermatogenesis) decreased during 15 and 60 days of exposure compared with their respective controls ($F_{(1.30)} = 23.680$; $p < 0.001$). No

significant differences were observed in the frequency of the other stages.

Table 4. Frequency of spermatogenic stages in seminiferous tubules of adult Swiss mice (n = 6 per group) treated for 15, 30, and 60 days with 0.35 mg/kg of GBH or water (control groups).

Parameters	Experimental groups					
	Control			GBH		
	15 days	30 days	60 days	15 days	30 days	60 days
Spermatogenic stages						
I–IV	25.0±4.0	29.0±5.0	28.0±3.0	31.0±5.0	36.0±5.0	35.0±4.0
V–VI	25.0±5.0	23.0±5.0	25.0±2.0	18.0±2.0*	18.0±3.0	18.0±5.0*
VII–VIII	27.0±5.0	26.0±3.0	27.0±4.0	27.0±6.0	23.0±5.0	23.0±7.0
IX	21.0±3.0	22.0±6.0	20.0±3.0	22.0±5.0	20.0±4.0	21.0±7.0
X–XI	4.0±2.0	2.0±1.0	3.0±1.0	4.0±2.0	4.0±1.0	4.0±1.0
XII	3.0±1.0	3.0±2.0	2.0±1.0	3.0±2.0	4.0±2.0	4.0±1.0

Data analyzed using two-way ANOVA followed by Tukey's post hoc test and expressed as mean ± standard deviation. *Significant difference between GBH-treated and control groups ($p < 0.05$). Stages I–IV (type A and intermediate spermatogonia, pachytene spermatocytes, and two generations of spermatids); stages V–VI (type A and type B spermatogonia, pachytene spermatocytes, and two generations of spermatids); stages VII–VIII (type A spermatogonia, preleptotene and pachytene spermatocytes, and two generations of spermatids); stage IX (type A spermatogonia, leptotene and pachytene spermatocytes, and one generation of spermatids); stages X–XI (type A spermatogonia, leptotene and zygotene spermatocytes, and one generation of spermatids); stage XII (meiosis I and II, with zygotene spermatocytes and one generation of spermatids). GBH: Glyphosate-based herbicide.

Spermatogenesis involves a complex network of hormonal interactions. Endocrine disruptors, such as glyphosate, may impair this process (CASALS-CASAS; DESVERGNE, 2011) locally through direct action on the testis or distantly by disrupting hormone release from the hypothalamus and pituitary, both essential for maintaining spermatogenesis (SMITH; WALKER, 2015).

Given the dynamic nature of spermatogenesis, data indicated that, despite the absence of significant differences, mice exposed to GBH showed an increased frequency of stages I–IV (24%), also classified as proliferative. This increase may explain the similar production of sperm between groups (Table 5) despite the significant reduction in stages V–VI. The epithelial dynamics is compensatory, with the epithelium adopting strategies in response to the nature of exposure.

Glyphosate exposure in rodents has been associated with damage to testicular architecture and cellular integrity. Studies have reported degenerative lesions, irregular arrangement of germ cells, and changes in seminiferous tubule diameter, including significant reductions in epithelial height (JIANG et al., 2018; HARITI et al., 2024). In addition, GBH may induce oxidative stress and apoptosis in the testes of exposed animals (JIANG et al., 2018; HASHIM et al., 2022). Glyphosate may also alter the permeability of the blood-testis barrier, a structure essential for maintaining proper spermatogenesis (GORGA et al., 2021).

DSP and sperm transit time through the epididymis

GBH did not change the number of mature spermatids in the testis or DSP (Table 5). Conversely, Nardi et al. (2017) observed a reduced number of testicular spermatids in Wistar rats treated with 50 mg/kg and 100 mg/kg of GBH for 35 days. However, the doses employed were considerably higher than those used in this study.

Table 5. Sperm count and daily sperm production (DSP) of adult Swiss mice (n = 8 per group) treated for 15, 30, and 60 days with 0.35 mg/kg of GBH or water (control groups).

Parameters	Experimental groups					
	Control			GBH		
	15 days	30 days	60 days	15 days	30 days	60 days
Testis						
MS (10^6 testis^{-1})	15.6±6.5	17.6±5.7	17.5±8.0	23.0±7.3	19.3±10.4	17.6±3.2
MS (10^6 g^{-1} testis^{-1})	126.8±49.4	159.8±37.4	150.2±71.6	178.0±63.7	153.8±59.8	140.6±18.6
DSP (10^6 testis^{-1} day^{-1})	3.2±1.4	3.6±1.2	3.6±1.7	4.7±1.5	4.0±2.2	3.6±0.7

Data analyzed using two-way ANOVA followed by Tukey's post hoc test and expressed as mean ± standard deviation. * Indicates a significant difference between GBH-treated groups ($p < 0.05$). GBH: Glyphosate-based herbicide; MS: Mature spermatids.

On the other hand, treatment with GBH induced changes in the epididymal parameters evaluated (Table 6). Sperm transit time through the caput/corpus segments was higher in the group treated for 15 days than in controls ($F_{(2.42)} = 4.364$; $p = 0.019$). Additionally, many spermatozoids were detected in the cauda segment of the group treated for 15 days ($F_{(2.42)} = 4.038$; $p = 0.025$). These findings require an integrated interpretation. The epididymis was divided into two segments due to distinct morphological and functional characteristics, despite both segments share important autocrine regulation to ensure proper sperm maturation. The accelerated transit through the caput/corpus directly influences the number of spermatozoa reaching the cauda within the same timeframe. Consequently, an increased number of spermatozoa was observed in the cauda epididymis of mice treated with GBH for 15 days.

Table 6. Sperm count and transit time through the epididymis of adult Swiss mice (n = 8 per group) treated for 15, 30, and 60 days with 0.35 mg/kg of GBH or water (control groups).

Parameters	Experimental groups					
	Control			GBH		
	15 days	30 days	60 days	15 days	30 days	60 days
Caput-corpus epididymis						
MS (10^6 epididymis^{-1})	5.0±4.2	2.8±1.4	4.1±2.1	3.5±1.5	3.4±1.9	3.5±1.0
MS (10^6 g^{-1} epididymis^{-1})	139.4±93.8	103.2±36.2	132.3±60.4	136.5±35.8	115.5±62.6	108.4±15.4
Sperm transit time (days)	1.4±0.6	0.8±0.1	1.2±0.6	0.7±0.2*	0.8±0.1	1.0±0.2
Cauda epididymis						
MS (10^6 epididymis^{-1})	3.0±0.4	2.7±1.1	4.3±2.7	4.6±2.9	3.3±2.7	4.0±0.9
MS (10^6 g^{-1} epididymis^{-1})	122.8±8.9	229.7±86.7	220.8±117.9	268.6±70.9*	228.4±127.3	216.7±30.8
Sperm transit time (days)	0.7±0.3	0.7±0.3	1.2±0.3	1.0±0.8	0.7±0.3	1.1±0.2

Data analyzed using two-way ANOVA followed by Tukey's post hoc test and expressed as mean ± standard deviation ($p < 0.05$). GBH: Glyphosate-based herbicide; MS: Mature spermatids.

The epididymis plays a critical role in the maturation and functional competence of spermatozoa. Changes in sperm transit time through the epididymis may impair the maturation process (CORNWALL, 2009). During this transit, the spermatozoid gradually acquires progressive motility and undergoes hyperactivation during capacitation in the female reproductive tract, enabling the acrosome reaction (SUAREZ; PACEY, 2006). *In vivo* alterations in sperm

motility of rodents treated with GBH have been reported by Jiang et al. (2018).

Moreover, the cauda epididymis stores mature and functionally competent spermatozoa until ejaculation (CORNWALL, 2009). Consequently, the less time the sperm remain in the caput/corpus, the greater the number of cells stored in the cauda. Notably, this alteration was not observed in mice treated with GBH for 30 or 60 days, reflecting a hormetic response to different exposure times.

Conclusion

Significant alterations in the stages of spermatogenesis and epididymal parameters (relative mass, transit time, and sperm count), particularly after 15 days of exposure to GBH, may indicate reproductive toxicity in mice, even at a dose equivalent to the ADI. However, exposure in this study involved a GBH (Gliz 480 SL), highlighting the need for further studies using isolated glyphosate or other commercial formulations to better elucidate reproductive toxicity in murine models.

Acknowledgments

The authors thank Professor Walter D. Júnior and Karina Simões, and research colleagues Monaliza L. Santos and Matheus Santos Costa for their valuable assistance in conducting this project. This study was supported by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) – Finance Code 001. T.C.R. and C.C.B. received support from the Goiás State Research Foundation (FAPEG); R.K.C. was supported by CAPES; M.L.A. was supported by the National Council for Scientific and Technological Development (CNPq – PQ1D); R.M.C. received support from the Tutorial Education Program/Secretariat of Higher Education – Ministry of Education (PET/SESu-MEC).

References

CASALS-CASAS, C.; DESVERGNE, B. Endocrine disruptors: from endocrine to metabolic disruption. **Annual Review of Physiology**, v. 73, p.135–162, 2011.

CORNWALL, G. A. New insights into epididymal biology and function. **Human Reproduction Update**, v. 15, p. 213–227, 2009.

COSTAS-FERREIRA, C.; DURÁN, R.; FARO, L. R. F. Toxic Effects of Glyphosate on the Nervous System: A Systematic Review. **International Journal of Molecular Sciences**, v. 23, p. 1–35, 2022.

DAI, P.; HU, P.; TANG, J.; LI, Y.; LI, C. Effect of glyphosate on reproductive organs in male rat. **Acta Histochemica**, v. 118, p. 519–526, 2016.

DE ARAUJO, L. G.; ZORDAN, D. F.; CELZARD, A.; FIERRO, V. Glyphosate uses, adverse effects and alternatives: focus on the current scenario in Brazil. **Environmental Geochemistry and Health**, v. 45, p. 9559–9582, 2023.

DILL, G. M.; SAMMONS, R. D.; FENG, P. C. C.; KOHN, F.; KRETZMER, K.; MEHRSHEIKH, A.; BLEEKE, M.; HONEGGER, J. L.; FARMER, D.; WRIGHT, D.; HAUPFEAR, E. A. **Glyphosate: discovery development, applications, and properties**. In: NANDULA, V. K. (Ed.). Glyphosate Resistance in Crops and Weeds. Hoboken: Wiley, 2010. p. 1–33.

GABRIELY, I.; MA, X. H.; YANG, X. M.; ATZMON, G.; RAJALA, M. W.; BERG, A. H.; SCHERER, P.; ROSSETTI, L.; BARZILAI. N. Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? **Diabetes**, v. 51, p. 2951–2958, 2002.

GORGA, A.; RINDONE, G. M.; CENTOLA, C. L.; SOBARZO, C. M.; PELLIZZARI, E. H.; CAMBEROS, M. D. C.; MARÍN-BRIGGILER, C. I.; COHEN, D. J.; RIERA, M. F.; GALARDO M. N.; MERONI, S. B. Low Doses of Glyphosate/Roundup Alter Blood-Testis Barrier Integrity in Juvenile Rats. **Frontiers in Endocrinology**, v. 12, p. 1–12, 2021.

HARITI, M.; KAMEL, A.; GHOZLANI, A.; DJENNANE, N.; DJENOUHAT, K.; AKSAS, K.; HAMOULI-SAÏD, Z. Disruption of spermatogenesis in testicular adult Wistar rats after short-term exposure to high dose of glyphosate

based-herbicide: Histopathological and biochemical changes. **Reproductive Biology**, v. 24, p. 1–10, 2024.

HASHIM, A. R.; BASHIR, D. W.; YASIN, N. A. E.; RASHAD, M. M.; EL-GHARBAWY, S. M. Ameliorative effect of N-acetylcysteine on the testicular tissue of adult male albino rats after glyphosate-based herbicide exposure. Journal of Biochemical and Molecular **Toxicology**, v. 36, p. 1–11, 2022.

HESS, R. A.; DE FRANCA, L. R. Spermatogenesis and cycle of the seminiferous epithelium. In: CHENG, C. Y. (Ed.). Molecular Mechanisms in Spermatogenesis. Advances in Experimental Medicine and Biology. New York: Springer, 2009. p. 1–15.

JIANG, X.; ZHANG, N.; YIN, L.; ZHANG, W. L.; HAN, F.; LIU, W. B.; CHEN, H. Q.; CAO, J.; LIU, J. Y. A commercial Roundup® formulation induced male germ cell apoptosis by promoting the expression of XAF1 in adult mice. **Toxicology Letters**, v. 296, p. 163–172, 2018.

KLEIN, H. S.; LUNA, F. V. **The Modernization of Brazilian Agriculture Since 1950**. In: KLEIN, H. S.; LUNA, F. V. (Eds.). Brazilian Crops in the Global Market. Palgrave Studies in Economic History. Menlo Park: Palgrave Macmillan, Cham, 2023. p. 5–38.

MATTSON, M. P. Hormesis defined. **Ageing Research Reviews**, v. 7, p. 1–7, 2008.

MAUER, M. M.; HARRIS, R. B.; BARTNESS, T. J. The regulation of total body fat: lessons learned from lipectomy studies. **Neuroscience & Biobehavioral Reviews**, v. 25, p. 15–28, 2001.

NARDI, J.; MORAS, P. B.; KOEPPE, C.; DALLEGRAVE, E.; LEAL, M. B.; ROSSATO-GRANDO, L. G. Prepubertal subchronic exposure to soy milk and glyphosate leads to endocrine disruption. **Food and Chemical Toxicology**, v, 100, p. 247–252, 2017.

PANDEY, A.; RUDRAIAH, M. Analysis of endocrine disruption effect of Roundup® in adrenal gland of male rats. **Toxicology Reports**, v. 2, p. 1075–1085, 2015.

PARADA-SIMÃO, T. R.; OLIVEIRA, F.; PACHECO, M. E.; ALVES, P. H. M.; LIBERTI, E. A. Effects of protein malnutrition on muscle fibers of the brachial biceps and medial pterygoid of Wistar rats. **Journal of Morphological Sciences**, v. 28, p. 196–203, 2011.

PHUSATE, V. T. Effect of herbicide based formulations on structure and function of testis in rats. **Indian Journal of Experimental Biology**, v. 60, p. 805–809, 2022.

RANA, I., NGUYEN, P. K.; RIGUTTO, G.; LOUIE, A.; LEE, J.; SMITH, M. T.; ZHANG, L. Mapping the key characteristics of carcinogens for glyphosate and its formulations: A systematic review. **Chemosphere**, v. 339, p. 1–20, 2023.

REAGAN-SHAW, S.; NIHAL, M.; AHMAD, N. Dose translation from animal to human studies revisited. **The FASEB Journal**, v. 22, p. 659–661, 2008.

SMITH, L. B.; WALKER, W. H. **Hormone signaling in the testis**. In: PLANT, T. M.; ZELEZNIK, A. J. (Eds.). Knobil and Neill's Physiology of Reproduction. 4 ed. Amsterdam: Elsevier, 2015. p. 637–690.

SOUZA, M. R.; CARVALHO, R. K.; CARVALHO, L. S.; SÁ, S.; ANDERSEN, M. L.; ARAÚJO, E. G.; MAZARO-COSTA, R. Effects of subchronic exposure to *Caryocar brasiliense* peel ethanolic extract on male reproductive functions in *Swiss* mice,
Reproductive Toxicology, v. 87, p. 118–124, 2019.

SUAREZ, S. S.; PACEY, A. A. Sperm transport in the female reproductive tract. **Human Reproduction Update**, v. 12, p. 23–37, 2006.

UMETSU, N.; SHIRAI, Y. Development of novel pesticides in the 21st century. **Journal of Pesticide Science**, v. 45, p. 54–74, 2020.

ZHAO, L.; ZHANG, J.; YANG, L.; ZHANG, H.; ZHANG, Y.; GAO, D.; JIANG, H.; LI, Y.; DONG, H.; MA, T.; WANG, X.; WU, M.; WANG, A.; JIN, Y.; YUAN, Y.; CHEN, H. Glyphosate exposure attenuates testosterone synthesis via NR1D1

inhibition of StAR expression in mouse Leydig cells. **Science of The Total Environment**, v. 785, p. 1–12, 2021.