https://doi.org/10.31925/farmacia.2025.6.1 **REVIEW**

EFFECTS OF PRENATAL AND NEONATAL EXPOSURE TO GLYPHOSATE AND GLYPHOSATE-BASED FORMULATIONS ON MALE AND FEMALE OFFSPRING FERTILITY AND REPRODUCTIVE ORGANS DEVELOPMENTAL EFFECTS

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Abstract

A systematic search (June 2025) of PubMed and ScienceDirect identified animal studies examining prenatal and lactational exposure to glyphosate or glyphosate-based herbicides (GBHs) and subsequent reproductive outcomes. Of 272 records screened, 58 full texts were assessed, and 10 met the inclusion criteria focusing on male or female fertility endpoints. Two studies reported effects in both sexes; five studies in male offspring (mice and rats) and seven in female offspring (rats and ewes). Evidence indicates endocrine-disrupting actions of glyphosate and GBHs in males, including impaired spermatogenesis and altered Leydig cell hormone regulation at low exposure levels. In females, very low doses reduced implantation sites and induced multigenerational effects in rats and ewes. Collectively, available data demonstrate that early-life glyphosate or GBH exposure can disrupt reproductive physiology across species.

Rezumat

O analiză sistematică a literaturii (iunie 2025), realizată în bazele de date PubMed și ScienceDirect, a identificat studii pe animale care au evaluat efectele expunerii prenatale și în perioada de lactație la glifosat sau la erbicide pe bază de glifosat (GBH), asupra funcției reproductive. Dintr-un total de 272 de articole identificate, 58 au fost analizate integral, iar 10 au îndeplinit criteriile de includere, vizând indicatori ai fertilității masculine și/sau feminine. Două studii au raportat efecte la ambele sexe, cinci au investigat descendenți de sex masculin (șoareci și șobolani), iar șapte descendenți de sex feminin (șobolani și oi). Rezultatele indică faptul că glifosatul și GBH-urile pot acționa ca perturbatori endocrini, în special la masculi, unde au fost observate afectarea spermatogenezei și modificări ale reglării hormonale a celulelor Leydig, chiar și la niveluri scăzute de expunere. La femele, doze foarte mici au determinat reducerea numărului de situsuri de implantare și apariția unor efecte multigenerațională la șobolani și oi. În ansamblu, datele disponibile arată că expunerea timpurie la glifosat sau la erbicide pe bază de glifosat poate perturba fiziologia reproductivă la diferite specii.

Keywords: glyphosate, fertility, endocrine-disrupting effects

Introduction

The global prevalence of female infertility has increased significantly since 1990, with notable disparities evident among different SDI regions and countries. The highest risk of infertility is observed among women aged 35 - 39, and there is an apparent trend towards an earlier onset of infertility [1].

It is well documented that exposure to chemicals in the uterine environment, early childhood, adolescence, or among previous generations can significantly affect the trajectory of risk for various disturbances in adulthood, particularly with respect to fertility in subsequent generations [2]. In certain instances, this trajectory indicates an association between the early-life risk factor and risk factor levels during pregnancy or at conception [3]. It has been demonstrated that chemical exposures experienced during pregnancy have the capacity to traverse the placenta. This results in the accumulation of these chemicals within the foetus [4]. Consequently, subsequent generations may be born with an innate predisposition to pollution-related adverse effects, a phenomenon attributed to exposure of the parental germ cells before conception and of the foetus to environmental contaminants during gestation [4]. Consequently, knowledge of early-life exposures is imperative for understanding disease progression, identifying individuals at high risk and determining the most effective interventions.

Glyphosate is among the most widely used herbicides globally. Its use has increased by more than 15-fold from 1996 to 2014, and this increase continues [5]. A plethora of issues surrounding glyphosate use has given rise to substantial political discourse in recent years. These include the spread of glyphosate-resistant weeds, concerns about health implications from occupational exposure or from residues on widely consumed foods, and broader environmental ramifications for biodiversity, water and soil [6]. The carcinogenic and reproductive effects of glyphosate and glyphosate-based formulations, as well as their detrimental impact on reproduction, have been observed in several studies [7, 8]. This issue is a primary point of discussion between regulatory agencies and research institutes. Despite numerous studies highlighting the potential developmental and reproductive toxicity of glyphosate, the most recent hazard assessment by the European Chemicals Agency (ECHA) in 2022 identified no critical areas of concern. However, specific data gaps were identified, leading to the conclusion that the previous classification of glyphosate as Eye Damage 1 and Aquatic Chronic 2 should be maintained, and that there is no justification for further classification as a carcinogen or a reproductive toxicant. In 2023, following evaluations conducted by the European Chemicals Agency (ECHA) and the European Food Safety Agency (EFSA), the European Commission (EC) renewed the approval of glyphosate for a further ten-year period, extending until 2033. This renewal was accompanied by the imposition of new conditions, including a prohibition on the pre-harvest use of glyphosate as a desiccant and a requirement for specific measures to protect non-target organisms [9]. In 2025, following the dissemination of a novel carcinogenicity study conducted by the Ramazzini Institute [10], EC petitioned ECHA, *via* the Committee for Risk Assessment, to reconsider the harmonised classification of glyphosate, and EFSA to reconsider the risk assessment based on the findings of the study above. To date, several studies have investigated the potential effects of glyphosate and glyphosate-based herbicides (GBHs) on the male and female reproductive systems [11-13]. These effects appear to be associated with the potential endocrine-disrupting properties of glyphosate and GBHs, which have been observed to induce disturbances in hormonal regulation, particularly during critical developmental phases such as prenatal and neonatal periods [14-16].

The present manuscript aims to evaluate all published studies on animal models to ascertain the effects of exposure to glyphosate and GBHs during pregnancy and lactation on fertility and reproductive outcomes in male and female offspring.

Materials and Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline 2020 [17] was utilised in the development of the present systematic review.

Study selection

A comprehensive search was conducted in June 2025 using PubMed (MEDLINE) and ScienceDirect to identify all published research studies to date. The following keywords were utilized in the search query: The search terms that were entered and which were then separated by the Boolean operator AND included the following: "prenatal exposure", "gestational exposure", "neonatal exposure", "glyphosate", "reproductive effects", "glyphosate-based pesticides", "GBHs", "male fertility", "female fertility", "reproductive outcomes" and "neonatal exposure". The initial search was conducted independently by two authors (ALH and AB), and any discrepancies were addressed through discussion. Subsequently, the search strategy was reviewed and discussed with a third author (AOD). Following the identification of relevant articles for inclusion, all authors convened to establish the inclusion criteria and devise a clear strategy for identifying pertinent additional articles. A critical evaluation of the dataset of studies was conducted by three authors (ALH, AB, LC). The initial exclusion of articles was based on their categorisation into one of the following classifications: book chapters, reviews or meta-analyses, conference abstracts, short communications, correspondence, or other non-relevant publications. In order to identify articles that describe preclinical research in which animals are exposed to glyphosate or GBHs during gestation or early neonatally, and in which this exposure determines male or female fertility or reproductive outcomes in offspring, a comprehensive analysis of the full texts of the remaining articles was conducted.

Data extraction

All the authors developed the data extraction template. Subsequently, two independent authors (AMH and AB) performed data extraction in duplicate; any discrepancies were discussed and resolved. Information on the study type, model, treatment duration, treatment dose, primary findings on male and female fertility, and reproductive toxicity was retrieved for analysis. The data were summarised in tabular form to facilitate effective presentation and interpretation.

Results and Discussion

Selection of the study

A systematic review of the two databases under consideration yielded 272 articles, comprising 66 in PubMed and 206 in ScienceDirect (Figure 1). Following the removal of 40 studies due to duplicate entries, the remaining 232 studies were screened by evaluating their titles and abstracts. Following a thorough examination of the extant literature, 174

articles were excluded on the grounds of irrelevance. The excluded articles comprised 68 book chapters, 59 review articles, 7 conference abstracts, 1 short communication, 1 correspondence and 38 other non-relevant publications (appendices, indices, contents). A total of 58 full articles were analysed; 26 were excluded because the exposure timeline differed from prenatal and early neonatal exposure, and 22 were excluded because other endpoints were evaluated in the study (Figure 1). A total of 10 articles satisfied the inclusion criteria for this systematic review (see Figure 1). As demonstrated in Figure 1, the selection of studies for inclusion was conducted in accordance with the 2020 PRISMA checklist [17].

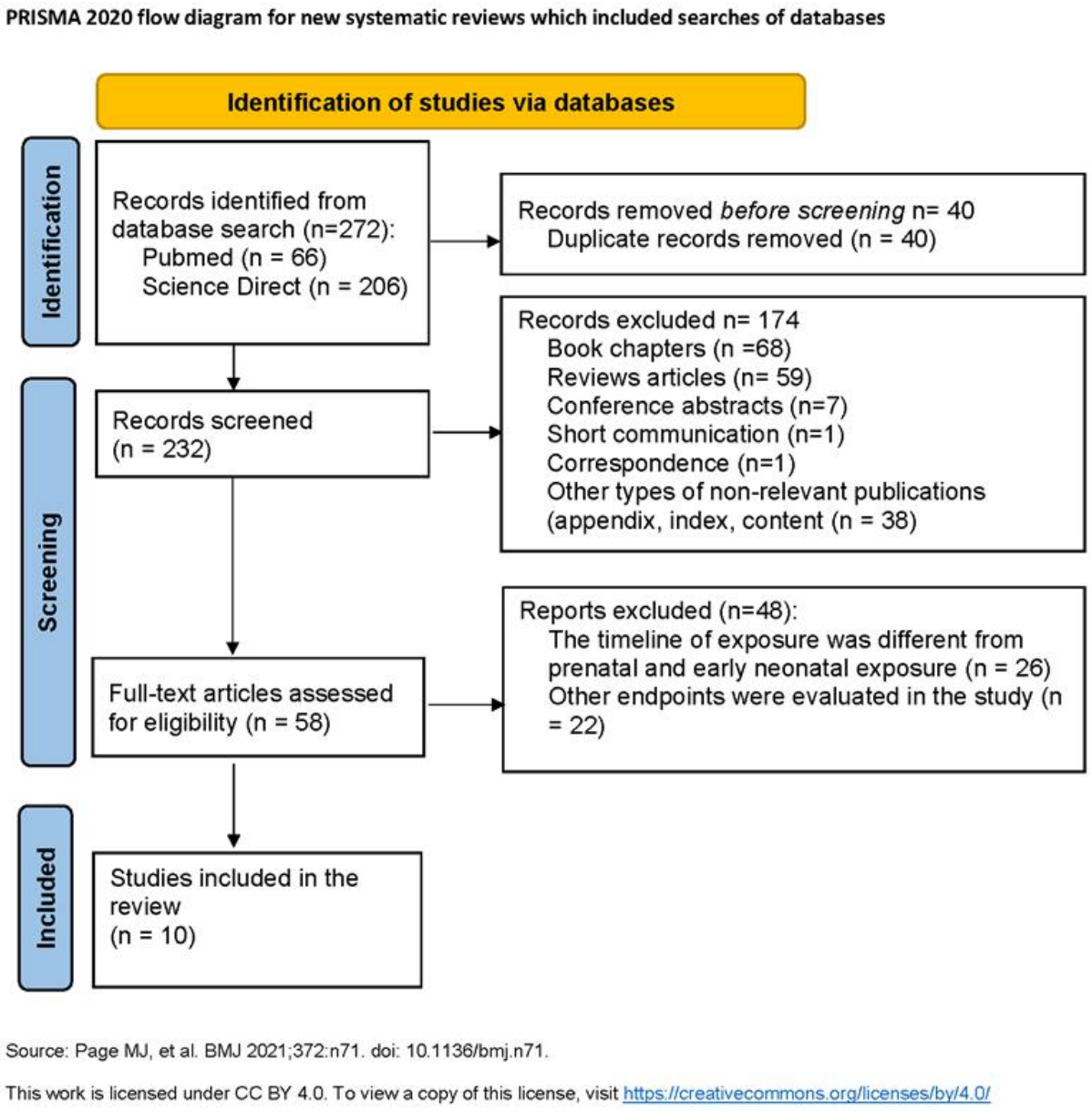

Figure 1.
The flowchart of selection of the studies included in the systematic review based on the 2020 PRISMA guidelines [17]

Study Characteristics

The main characteristics of the studies included in the systematic review are presented in Table I. The systematic review included three studies that evaluated pre- and post-natal exposure to glyphosate and GBHs on male fertility and reproduction outcomes. These five studies evaluated pre- and postnatal exposure to glyphosate and GBHs on female fertility and reproduction outcomes, and two studies evaluated pre- and postnatal exposure to glyphosate and GBHs on both male and female fertility and reproduction outcomes.

Table I

Characteristics of the studies included in the systematic review

Model/Type of exposure	Treatment	Doses	Adverse effects on sexual function and fertility (#)	Reference
Male				
Mice studies				
Swiss mice pregnant females were treated from E10.5* to PND20 (F0) F1 males were evaluated PND5, PND20, PND35 and 8 months old adults	Purified glyphosate (G) or GBH (Roundup®3 Plus) was administered orally in drinking water	Equivalent to 0.5, 5 and 50 mg/kg/day glyphosate based on average water uptake	**Dams**: - No evaluation was done regarding the maternal toxicity **F1 Male**: - ↑ BW (PND20) in G0.5 and GHB5 - ↓ relative testis weight G0.5 and G5; - ↓ epididymis weight GBH0.5; - ↓ seminal vesicle weights G5, G50 and GBH0.5 (PND35) - ↓ spermatozoa in total epididymis G5 and GBH0.5 (PND35) - ↓ serum testosterone G0.5 and G50 (PND35) - altered testis morphology in G groups (PND20), reversible effects till PND 35 - ↓ number of ZBTB16 positive cells (PND 35) G5 - ↑ Bax and Bcl2 gene expression involved in apoptosis (G groups) and Nanos3 (G0.5, G50) and Foxo1 (G05) (PND5) - ↓ Kit expression (G50) and Sall4 expression (all G groups) (PND5) **Long-term effects (8-month-old F1 males)**: - ↓ relative testis weight (G0.5; GHB5) - ↓ testosterone level (GBH 5, GBH 50)	[18]
C57BI/6 pregnant female mice were treated from GD4 till PND30 (weaning) (F0) F1 males were kept without treatment from PND30 till PND150	GBH (Roundup Original DI® (Monsanto, Brazil) in drinking water	0.5% - similar to the level of groundwater after agricultural practice Equivalent to 420 mg/kg bw/day glyphosate	**Dams:** - ↓ BW gain during gestation, - ↓ BW at PND30 **F1 Male**: - delayed testicular descent (not associated with changes in body mass, BWs not changed) (at PND150, no changes between the groups in relative or absolute weights of testis, epididymis, seminal vesicle and prostate); - ↓ number of spermatozoa in the cauda epididymis - ↓ epithelial height of the seminiferous epithelium - ↑ intratesticular testosterone conc. - ↑ plasma luteinizing hormone (LH) (PND150) and - ↑ β-LH pituitary protein level	[19]
Rats studies				
Wistar rats, pregnant females were treated from GD0 till PND21 (weaning) (F0) F1 males were kept without treatment from PND22 till PND140	GBH (Roundup®, Monsanto, Brazil) in drinking water	0, 50, 150 and 450 mg/kg bw	**Dams:** no sign of maternal toxicity identified, no changes in litter size, number of live/dead pups, or sex ratio **F1 Male**: - the preputial separation was advanced in the HD group, but in the HC data - ↑ abnormal sperm (puberty); ↓ daily sperm production and sperm number (during adulthood). - Dose-dependent ↓ testosterone levels (puberty) - Increased degeneration of the testis (puberty and adulthood in MD and HD groups)	[20]
Wistar rats, pregnant females, were treated from GD18-PND5 (F0) F1 males were kept without treatment till PND 60	GBH (Roundup Transorb, Monsanto Brazil) administered orally, by gavage once *per* day	50 mg/kg/day	- ↓ puberty onset - ↑ preference for the female gender, ↑ latency to first mount, latency to first intromission - ↑ testosterone, ↑ oestradiol levels - ↑ LH mRNA and ↑ protein levels in pituitary and serum - ↑ FSH mRNA expression - ↑ sperm production - ↑ weight of drained seminal vesicle	[21]

Model/Type of exposure	Treatment	Doses	Adverse effects on sexual function and fertility (#)	Reference
SD rats pregnant females were treated from GD6-PND28 (F0) F1 males were treated until PND73 ± 2 or PND 125 ± 2	Analytical grade glyphosate (> 99.5% purity) or GBH (Roundup Bioflow, Italia) administered orally in drinking water	1.75 mg/kg/day	**Dams**: no sign of maternal toxicity identified **F1 Male**: - ↑ AGD (G and GBH) - ↓ serum DHT levels in GBH - ↓ fT/TT ratio in G (6 weeks) and GBH (13 weeks) - ↓ DHT/TT ratio GBH (13-weeks)	[22]
Female				
Rats				
Wistar rats, pregnant females were treated from GD0 till PND21 (weaning) (F0) F1 females were kept without treatment from PND22 till PND140	GBH (Roundup®, Monsanto, Brazil) administered orally in drinking water	0, 50, 150 and 450 mg/kg bw	**Dams:** no sign of maternal toxicity identified, no changes in litter size, number of live/dead pups, or sex ratio **F1 Females**: - the vaginal canal opening was delayed (all doses)	[20]
Wistar rats, pregnant females, were allowed to reproduce without treatment (F0) F1 females were treated on PND1, 3,5 and 7 in the nape of the neck and evaluated in PND8 and PND2, while others were weaned on PND21 and kept till adulthood, mated with males on PND90 and then sacrificed in GD 9	GBH (containing 54% glyphosate) was administered sc. injection	2 mg/kg bw/day	**F1 females PND 8**: - ↑ Wnt5a (luminal epithelium) - ↑ β-catenin (luminal epithelium) **F1 females PND21**: - ↑ Wnt5a (subepithelial stroma) - ↑ β-catenin (stromal compartment) - ↓ β-catenin (glandular epithelium) **F1 females GD9** - ↓ Wnt5a (AM zone) - ↓ Wnt7a (luminal epithelium of the M zone) - ↑ Dkk1 mRNA - ↓ sFRP4 mRNA	[23]
Wistar rats, pregnant females were treated from GD9 till PND21 (weaning) (F0) F1 females were kept without treatment from PND22 till PND90, when they mated with males and were evaluated till GD19	GBH (MAGNUM SUPER II, Argentina) (54% glyphosate) administered orally in food (pellet chow)	2 mg/kg bw/ day, 200 mg/kg bw/day based on the food consumption - real obtained doses in F0 were: 3.69 ± 0.07 mg/kg bw/day and 352.2 ± 4.78 mg/kg bw/day	**Dams (F0):** ↑ relative BW loose (LD1 → PND21) in (GBH-LD group) **F1 females (GD19):** - ↓ implantation sites - ↑ pre-implantation loss **F2 offspring (GD19):** - ↓ Foetal body weight - ↓ Foetal body length - ↑ Placental weight (HD only) - ↑ Placental index (HD only) - ↑ Foetal malformation (HD only)	[24]
SD rats pregnant females were treated from GD6-PND28 (F0) F1 females were treated until PND73 ± 2 or PND 125 ± 2	Analytical grade glyphosate (> 99.5% purity) or GBH (Roundup Bioflow, Italia) administered orally in drinking water	1.75 mg/kg/day	**Dams**: no sign of maternal toxicity identified. **F1 Female**: - ↑ AGD (PND4) (GBH) - ↑ age of first oestrus (GBH) - ↑ TT (GBH) (13weeks) - ↑ fT index (TT/SHBG) (GBH) - ↓ DHT/TT ratio (GBH) (13weeks)	[22]
Wistar rats, pregnant females, were treated from GD9-PND21 (F0) F1 females were kept without treatment from PND22 till PND90, when they mated with males and were evaluated till GD19	Analytical grade glyphosate (96% purity) or GBH (Magnum Super II, Grupo Agro S.R.L.) administered orally in food (pellet chow)	2 mg/kg bw/ day based on the food consumption - real obtained doses in F0 were: 3.94 ± 0.11 mg/kg bw/day (G) and 3.75 ± 0.10 mg/kg bw/day (GBH)	**Dams**: no sign of maternal toxicity identified. **F1 females:** - ↓ implantation sites (both groups) (GD19) - ↑ percentage of preimplantation loss (both groups) (GD19) - ↑ E2 serum levels (both groups) (GD5) - ↑ uterine ERα protein (both groups) (GD5) without difference in Erα mRNA levels - ↓ PR mRNA (G)(GD5) - ↓ Hoxa10 and Lif genes (both groups) (GD5)	[25]

Model/Type of exposure	Treatment	Doses	Adverse effects on sexual function and fertility (#)	Reference
Ewe				
Friesian ewe pregnant females were allowed to reproduce without treatment (F0) F1 females were treated from PND1 to PND14 by sc, injection in the nape of the neck and evaluated in PND45	GBH (Roundup Full II® (Argos SRL, Argentina) (containing 54 % glyphosate) administered by sc. injection	2 mg/kg bw/day	**F1 females PND 45:** - ↓ cell proliferation - ↑ p27 protein expression - ↑ IGFBP-3 gene expression - ↓ ERα expression (luminal and glandular epithelium and subepithelial stroma) - ↓ PR expression (luminal epithelium) - ↑ PR expression (glandular epithelium and subepithelial stroma) - ↓ Wnt5a (glandular epithelium) - ↓ Wnt7a (subepithelial stroma) - ↓ β-catenin (luminal epithelium, glandular epithelium) - ↓ Hoxa 10 (subepithelial stroma) - ↓ Foxa2 (glandular epithelium)	[26]
Corriedale ewe pregnant females were allowed to reproduce without treatment (F0) F1 females were treated from PND1 till PND14 orally	GBH (Roundup Full II® (Argos SRL, Argentina) (containing 54 % glyphosate) administered orally	1 mg/kg bw/day glyphosate and 50 mg/kg bw/day pFSH from PND41 till PND 43	**F1 females (GBH treated):** - ↓ ERα expression - ↓ PR expression - ↓ ACVRII - ↓ MBP15 mRNA - ↓ Anti-Mullerian hormone expression (primordial and antral follicles) - ↓ Bone morphogenetic protein four expression (primordial follicles) **F1 females (GBH + pFSH treated)**: - ↓ ACVRII - ↓ MBP15 mRNA - ↓ FSHr	[27]

[#] all presented parameters were statistical significant ($p < 0.05$); GD – gestational day; PND – post-natal day; E – embryonic day; * the day of vaginal detection was considered embryonic day; HD – high dose; MD – medium dose; HC – historical control data; AGD – anogenital distance; DHT – 5α-dihydrotestosterone; TT – total testosterone; fT – free testosterone; M zone – mesometrial; AM zone – anti-mesometrial; GBH-LD – low dose group treated with GBH; SHBG – sex-hormone binding globulin; E2 – oestradiol; Erα – oestrogen receptor alpha; PR – progesterone receptor; pFSH – porcine follicle-stimulating hormone; ACVRII – activin receptor II; BMP15 – bone morphogenetic protein 15; FSHr – follicle stimulating hormone receptor

A total of 10 studies were identified, two of which presented effects relevant for both male and female reproductive and fertility outcomes. Of these, five studies reported effects on the reproductive system in male offspring, two in mice [18, 19] and three in rats [20-22] and seven studies reported effects on the reproductive system of female offspring, 5 in rats [20, 22-25] and 2 in ewe [26, 27] (Table I).

Effects of glyphosate and GBH on the male reproductive system

Only two studies evaluated the pure glyphosate effects in comparison with those of GBH on reproductive outcomes in males [18, 22], while three studies evaluated the effects of GBH [19-21].

In mice, Lorenz *et al*. [18] evaluated the comparative effects of glyphosate and GBH (Roundup®3 Plus) exposure during gestation and lactation on the male reproductive system, using very low doses (approximately environmentally relevant doses for humans). This study demonstrated that glyphosate, but not GBH, decreased testicular weights at PND35 and in adult-hood [18]. Furthermore, both glyphosate and GBH have been shown to reduce seminal vesicle weight at specific doses, suggesting that these substances may act as endocrine disruptors. This phenomenon was concomitant with a decline in blood testosterone levels, as observed in the glyphosate-treated subjects at PND 35 and in the GBH-exposed subjects after 8 months [18]. It was observed that the effects evidenced in this study did not conform to a monotonic dose-response curve, with several effects being more pronounced at the low dose than at the high dose. This finding is consistent with recent studies on endocrine-disrupting chemicals (EDCs), which have demonstrated the capacity to influence endocrine responses at extremely low hormone concentrations [28]. An investigation was conducted into the potential toxicological impact of glyphosate and GBH on spermatogenesis at the gene level. The increase in gene expression linked to apoptosis during early spermatogenesis – for example, Bcl-2 and Bax in animals exposed to glyphosate [18] – could result in an imbalance between spermatogonial and Sertoli cells. This balance is imperative for the differentiation and development of the seminiferous tubules [29]. Glyphosate has been shown to reduce KIT and Sall4 expression at PND5, which are pivotal to germ cell differentiation [30]. Concurrently, the investigation revealed that glyphosate augmented Nanos3 and Foxo1 mRNA expression. The latter is implicated in the

maintenance and self-renewal of spermatogonial stem cells [31]. The net effect of these changes, as postulated by the present study, is a decrease in spermatozoa and morphological defects observed at PND20 and in adults [18].

Teleken *et al*. [19] evaluated the possible male adverse reproductive outcomes in mice exposed to GBH (Roundup®) during pregnancy and lactation, at higher doses compared to the first study [18], but below the NOAEL for glyphosate. These effects appear to be independent of maternal toxicity, as only slight modifications were observed in body weight (BW) and BW gain. Additionally, no effects were observed on gestation length or pup viability [19]. The principal effects of the male reproductive system are manifested as a retardation of testicular descent. Concurrently, a decline in spermatozoa within the cauda epididymis was observed. Furthermore, a diminution in epithelial height of the seminiferous tubules was evident, along with an escalation in intratesticular testosterone concentrations and an increase in plasma and pituitary LH levels [19]. The effects observed regarding testicular descent may be associated with the endocrine characteristics of GBHs, which appear to interfere with the hormonal regulation performed by the Leydig cells during the process of descent [32]. Furthermore, the repercussions on spermatozoa and hormone levels can be associated with the properties of GBH, which have been demonstrated to disrupt spermatogenesis. This process is contingent on LH levels, which in turn stimulate Leydig cells to produce testosterone [33].

In rats, the effects observed on the decrease of testosterone levels and reduced sperm production during adulthood were similar to those seen in mice. This decrease in testosterone levels and reduction in sperm production occurred in a dose-dependent manner following exposure to GBH [20]. Observations of changes in adult subjects following exposure during the sensitive period of foetal development support the hypothesis that environmental pollutants can induce epigenetic changes that may contribute to the later development of disease [34]. The impact of GBH on sperm quantity and quality may be attributed to its dose-dependent effect on reducing testosterone levels, attributable to GBH's endocrine-disrupting properties. The functional activity of spermatogenesis and sperm maturation is under the regulatory control of testosterone [35].

Romano *et al*. demonstrated contradictory outcomes, reporting elevated sperm production and testosterone levels after prenatal exposure to GBH [21], in contrast to the findings of prior studies conducted on rats [20] and mice [18]. These disparities are attributable to the study's exposure window, as reported by Romano *et al*. [21] the exposure of animals occurred during the perinatal period, coinciding with the onset of hypothalamic sexual differentiation. In contrast, in previous studies, the exposure persisted into the prepubertal period. It has been demonstrated that exposure to glyphosate or GBH during the prepubertal period can induce anti-androgenic effects, which are concomitant with a decline in testosterone levels [36]. Furthermore, the impact on sperm quantity is associated with the number of Sertoli cells formed during the prepubertal period, influenced by FSH, which determines the future dimensions of the testes and future sperm production [37].

A recent study conducted by the Ramazzini Institute used low doses of glyphosate and GBH, well below acceptable daily intake levels. This investigation revealed that these substances affected sexual maturation in F1 males, both before and during the pubertal period. Notably, these effects differed between males and females [22]. The principal observations recorded in the male subjects pertained to elevated AGD and a disruption in androgen metabolism, which culminated in a diminished conversion of testosterone to dihydro-testosterone (DHT) after GBH exposure. This phenomenon is presumably attributable to reduced 5α-reductase activity [22].

Effects of glyphosate and GBH on the female reproductive system

Only two studies evaluated the effects of pure glyphosate compared with GBH on reproductive outcomes in females [22, 25], whereas five studies evaluated the effects of GBH [20, 23, 24, 26, 27]. In rats, as Ingaramo *et al*. [23] have demonstrated, exposure to GBH during the neonatal period has the capacity to induce impaired fertility by modulating the uterine expression of Wnt5a and WNT7a and β-catenin in peripubertal and adult pregnant rats. As previously demonstrated, Wnt5a overexpression interferes with critical aspects of embryo implantation, thereby reducing fertility [38]. The effects under discussion, as determined by GBH, are consistent with the estrogenic effects of glyphosate [39, 40] and are consistent with findings that, after estradiol injection, differential cell-specific upregulation of Wnt5a mRNA was observed [41]. These effects are supported by the findings of Milesi *et al*. [24] that examined the effects of in utero and lactational oral exposure to low doses of GBH on the development and reproductive outcomes of F1 and F2 generations. GBH was administered using a modified food pellet chow containing GBH. No maternal toxicity or toxic effects on F1 offspring were observed after exposure till approximately 350 mg/kg bw/day GBH [24]. In relation to the implications for reproduction and fertility arising from prenatal and lactational exposure to GBH on F1 females, a decline in implantation sites has been observed in a dose-dependent manner and an increase in pre-implantation embryo loss [24]. The absence of variation in the number of corpora lutea indicates that the observed effects are unlikely to be due to impaired ovulation. Instead, they may be attributable to GBH-induced alterations in uterine organogenesis during embryonic

development, preventing the uterus from reaching the receptive state necessary for implantation in adulthood. Alternatively, they could be related to reduced oocyte fertilisation, impaired embryo development, or defects in tubal transport [42]. A further intriguing discovery from this study concerns the developmental effects observed in F2 offspring, which indicate a mechanism of transgenerational induction of congenital anomalies by GBH. This finding aligns with the theory that numerous environmental toxicants can modify chromosomal or epigenetic information in the absence of direct exposure, thereby resulting in transgenerational inheritance of disease [43]. In a subsequent study, the same group of researchers evaluated both glyphosate and GBH to elucidate the mechanism underlying impaired embryo implantation in female rats treated with either [25]. The study demonstrated that the administration of glyphosate and GBH disrupted serum E2-to-progesterone ratios, a pattern associated with dysregulation of ERα and PR uterine expression. The research further showed a decrease in the expression of their downstream hormone-responsive genes, including Lif and Hoxa10. These alterations occurred during the preimplantation period, a time frame that can influence the reduction in the window of implantation [25].

The endocrine-disrupting properties of GBH are further substantiated by the findings of the Ramazzini Institute study [22], which demonstrated that very low doses of GBH elicited androgen-mediated effects in female subjects, manifesting as an increase in AGD and first oestrous age. The present finding is corroborated by an increase in total testosterone and TT/SHBG, alongside a decrease in the DHT/TT ratio, a sign of altered testosterone metabolism [22].

Taking into consideration the similarity between uterine development in women and sheep, compared to rodents [44], and that in both species, the gland development of the primary postnatal uterine process [45], Alarcon *et al*. [26] evaluated the effects of neonatal GBH exposure on sheep uterine development. They showed that exposing ewe lambs to low levels of GBH during the neonatal period affected uterine cell growth and the expression of markers of uterine development and differentiation, including β-catenin, Wnt7a, Wnt5a, Hoxa10, Foxa2 and steroid receptors. Furthermore, other studies have shown that these molecules undergo alterations in the prepubertal uterus of neonatally GBH-exposed rats [25]. Despite the divergent patterns of expression exhibited by Hoxa10 and Wnt7a in the uterus of rats and ewe lambs, the estrogenic agonist effects of GBH (illustrated by increased PR expression) are analogous in both species. Additional studies are required to elucidate these variations. Furthermore, it is important to note that the observed effects may vary depending on the brand and/or the commercial formulation of glyphosate used. A new study from the same research group aimed to evaluate the impact of neonatal GBH exposure on gonadotropin (FSH)- super-stimulated ewe lambs [27]. The study demonstrated that neonatal GBH exposure disrupts key molecular mediators of follicular development, thereby impairing FSH action at ovarian receptors and reducing ovarian reserve and female fertility (see Table I) [27].

In the present systematic review, only two studies compared the effects of glyphosate and GBH on male reproductive outcomes [18, 22], and two studies examined female reproductive outcomes [22, 25]. The remaining studies primarily concentrated on the effects of GBH. Despite numerous studies reporting that the deleterious effects of GBH are more potent than those of glyphosate alone [46, 47], Pham *et al*. [18] found that pure glyphosate had a greater effect on the male reproductive system than GBH. It can be posited that the observed effects are due to the greater water solubility of the isopropylamine salt in GBH. This enhanced solubility facilitates faster and more efficient urinary excretion, consistent with its lower systemic toxicity relative to glyphosate acid [46]. Lorenz *et al*. [25] demonstrated that both glyphosate and GBH induce comparable hormonal and uterine changes in female rats. This finding suggests that glyphosate is predominantly responsible for the observed adverse effects. In contrast, Manservisi *et al*. [22] demonstrated that GBH exhibited more potent effects on both male and female reproductive outcomes than glyphosate alone. This finding is attributed to the potential endocrine-disrupting properties of glyphosate and its formulations. The observed effects corroborate the results of earlier mixture studies. The findings suggest that the active ingredient in glyphosate and components of formulations may have a cumulative impact on endocrine-sensitive endpoints [47]. A potential explanation for observed discrepancies between studies on glyphosate and GBH is variation in GBH formulations among manufacturers. It has been hypothesised that the novel generation of co-formulants is less deleterious than their predecessors [48].

A limitation of this study is the heterogeneity of GBHs across studies. This substance varies across studies, and the co-formulants have not yet been tested individually to identify the most appropriate formulation with reduced toxicity. The second limitation concerns the varying doses used in the identified studies, which fall between the acceptable daily intake for humans and the no-observed-adverse-effect level (NOAEL) for animals. Despite the variability in comparison levels, the effects associated with the endocrine-disrupting properties of glyphosate and GBH are consistently observed across studies.

Conclusions

Research involving male animals has demonstrated endocrine-disrupting properties of glyphosate and GBH following prenatal and early postnatal exposure. These properties have been shown to affect the reproductive system, resulting in impaired spermatogenesis and impaired Leydig cell hormone regulation in both mice and rats, even at very low environmentally relevant doses of glyphosate and GBH. In the context of female animal studies, glyphosate and GBH have been observed to demonstrate androgen-mediated effects, resulting in impaired fertility through a reduction in implantation sites. Of particular interest is the transgenerational inheritance of these effects, which have been observed in the second generation of offspring following exposure during the prenatal and lactational periods. The effects were consistent in rats and ewe models at very low environmentally relevant doses of glyphosate and GBH. The present findings emphasise the potential health implications for both animal and human populations associated with glyphosate and GBH, particularly regarding fertility, arising from endocrine disruption. Consequently, a thorough reanalysis of existing data by regulatory bodies is imperative to protect human health and the environment.

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Conflict of interest

The authors declare no conflict of interest.

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