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Effects of metformin and glutathione alone and in coformulation with other compounds on reproductive health and fertility in diabetic male rodents: A systematic review

Razia Sardar^{1,2}, Fatima Sardar^{1,2}, Nor–Ashikin Mohamed Noor Khan^{1,2✉}, Fathiah Abdullah^{2,3}, Yuhaniza Shafinie Kamsani^{1,2}¹Faculty of Medicine, Universiti Teknologi MARA, Selangor Branch, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia²Maternofetal and Embryo (MatE) Research Group, Universiti Teknologi MARA, Selangor Branch, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia³Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch, Tapah Campus, 35400 Tapah Road, Perak, Malaysia

ABSTRACT

Objective: To systematically review the effects of administering metformin and glutathione alone and in coformulation with other compounds on the fertility and reproductive health of diabetic male rodents.

Methods: The guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) were followed to conduct this systematic review. Studies published until August 2024 in PubMed, Web of Science, and Scopus databases were searched, identified, screened, and selected for a detailed review. The keywords included metformin, diabetes, reproduction, glutathione, and rodent models.

Results: A total of 166 studies were identified, of which 11 met the inclusion criteria and were included in the qualitative synthesis. One additional study was identified through snowballing and citation tracking, bringing the total to 12 studies. The findings indicate that metformin and glutathione, administered alone or in combination with other compounds, improved sperm count, motility, and morphology; restored reproductive hormone levels; reduced oxidative stress markers; and improved testicular histopathology in diabetic male rodents.

Conclusions: Coformulation of metformin and glutathione with other compounds was found to be more effective in improving fertility and reproductive parameters in diabetic male rodents compared to mono-administration. However, further studies on the coformulation of metformin and glutathione are needed to confirm their efficacy and elucidate the underlying mechanisms.

Study registration: The study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) with registration number CRD42024561820.

KEYWORDS: Diabetes; Metformin; Glutathione; Coformulation; Reproductive health; Infertility

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycaemia and resulting from an insulin

Key Points

Question: What are the effects of metformin and glutathione alone and in coformulation with other compounds on the fertility and reproductive health of the diabetic male rodents?

Findings: Coformulation of metformin and glutathione was associated with improvements in sperm count, motility, and morphology; restoration of reproductive hormone levels; reduction of oxidative stress markers; and improvement in testicular histopathology in diabetic male rodents.

Meaning: Metformin and glutathione alone and with other compounds may improve male reproductive function under diabetic conditions. However, current data are insufficient; hence, further studies are needed to validate efficacy and clarify mechanisms.

✉To whom correspondence may be addressed. E-mail: noras011@uitm.edu.my

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secretion defect. It is a rapidly growing disease that leads to a poor quality of life[1]. Persistent hyperglycaemia in DM leads to excess production of reactive oxygen species (ROS), instigating testicular damage and resulting in lower fertility[2]. The International Diabetes Federation estimated that 415 million individuals worldwide had DM in 2015[3]. In 2017, 8.8% of the global population was affected by DM, and this is expected to rise to 9.9% (629 million) by 2045[4].

High blood glucose levels can trigger oxidative stress (OS), a state of imbalance between oxidation and the antioxidant defence system in the body[5]. Hyperglycaemia in the testis and epididymis can cause free radicals that damage sperm[6]. An increased ROS level primarily causes diabetes-induced male infertility, as low ROS levels positively regulate sperm function[7]. DM affects reproductive health through reduced serum testosterone levels, impaired erection and ejaculation, spermatogenesis, and a decrease in Leydig cells[8], leading to reduced fertility[9]. DM negatively impacts sperm production, motility, viability, increases aberrant morphology, DNA fragmentation, and reproductive potential[10].

Metformin (Met) is an oral anti-diabetic drug prescribed widely for treating type 2 diabetes mellitus (DM- II) by increasing tissue sensitivity to insulin and glucose, and may also improve blood sugar control in patients with type 1 diabetes mellitus (DM- I) [11]. Met has been used in clinics for nearly 60 years to help patients with diabetes manage their blood glucose levels[12,13]. Prolonged Met treatment enhances sperm fertilization capability by promoting spermatogenesis in male diabetic rats[14].

Glutathione (GSH) is the mother of all antioxidants, which preserves the reduced forms of exogenous antioxidants like vitamins C and E while actively participating in the neutralization of free radicals and ROS[15]. GSH, a natural antioxidant found in humans, animals, fungi, and bacteria, is crucial in the body's nonenzymatic antioxidant defence system and acts as an excellent free radical scavenger[16]. It prevents oxygen production, scavenges superoxide, and protects against lipid peroxidation in the plasma membrane[17].

GSH has the potential to delay the onset of diabetic complications, rectify metabolic irregularities, and provide protection against OS damage caused by ROS[18]. GSH, the most abundant intracellular non-enzymatic antioxidant, plays a critical role in maintaining redox homeostasis. It is crucial in the male reproductive system, where it supports sperm motility, capacitation, and acrosome reaction[19,20]. Antioxidant regimens may be effective in combating male factor infertility caused by OS[21]. GSH supplementation has been shown to improve sperm quality in infertile males with varicocele or urinary tract inflammation[22].

At present, there are various clinical interventions for diabetes, including the administration of oral hypoglycaemic drugs. However, several demerits accompany oral drugs, such as weight gain, diarrhea, skin allergies, and, most importantly, reduced bioavailability and resistance to drugs with long-term use. Recent

research has focused more on finding alternative strategies, including the use of antioxidants; however, the mechanisms underlying their protective effects remain unclear.

This systematic review evaluates the effects of administering Met and GSH alone and in combination with other compounds on male reproductive health in diabetic rodent models. It is crucial to discover novel antioxidants with anti-diabetic properties that are safe and effective for improving male reproductive health, highlighting their potential clinical relevance for managing diabetes-associated male infertility.

2. Methods

2.1. Protocol registration

The protocol for this systematic review, focusing on the effect of administering Met and GSH alone and in combination with other compounds on reproductive health and fertility, was registered with PROSPERO (CRD42024561820). The registered protocol is being followed for the step-by-step methodology of this study.

2.2. Research question formulation

The research question (RQ) of this study was formulated using the PICO (Population, Intervention, Comparison, Outcomes) framework. The population (P) comprised diabetic male rodents. The intervention (I) involved the administration of Met and GSH, either alone or in coformulation with other compounds. Comparison (C) focused on reproductive health and fertility, and outcomes (O) were assessed to evaluate the improvements of these interventions. This review addressed the following research question: “What is the role of administering Met and GSH, alone or in combination with other compounds, in improving reproductive health and fertility in diabetic male rodents?”

2.3. Search strategy

The PubMed, Scopus, and Web of Science (WoS) databases were searched using four combinations of Medical Subject Headings (MeSH) keywords (presented in Table 1 in the “Results” section). The keywords included metformin, diabetes, reproduction, glutathione, and rodent models. The aim was to identify studies that fell within the scope of this review. The initial search was conducted for studies published until August 2024. In addition, articles found through random search, snowballing, and citation tracking were also included to summarize the work conducted. The eligibility of each identified study was evaluated by screening the title, abstract, and full text following the inclusion and exclusion criteria.

2.4. Inclusion and exclusion criteria

The systematic review protocol was established using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. The original research studies that addressed the effects of administering Met and GSH alone and in co-administration with other compounds on fertility and reproductive parameters in diabetic male rodent models were included. Studies without original research, reviews, mini-reviews, expert opinions, and reports were excluded. Studies that focused on the effects of administering Met and GSH alone and in combination with other compounds, other than reproduction and fertility parameters, or having rodent models without diabetes, were also excluded. The articles' inclusion was limited to full-text availability in English and published until August 2024.

2.5. Data extraction

The included studies were reviewed to extract data related to the

type of model, sample size, type of drug, dosing interventions, parameters measured against pre-defined objectives, methodological steps, findings, and contextual factors. This was done using a data charting form developed by RS and FS, and shared on a platform where all authors have access to review, edit, and update data records after reviewing the included articles.

3. Results

3.1. Search outcomes

The details of identified, screened, eligible, and included studies at each step of the selection of studies for qualitative synthesis are shown in Figure 1. The number of studies identified from PubMed, Scopus, and WoS databases for each combination of keywords is shown in Table 1. A total of one hundred and sixty-six records were

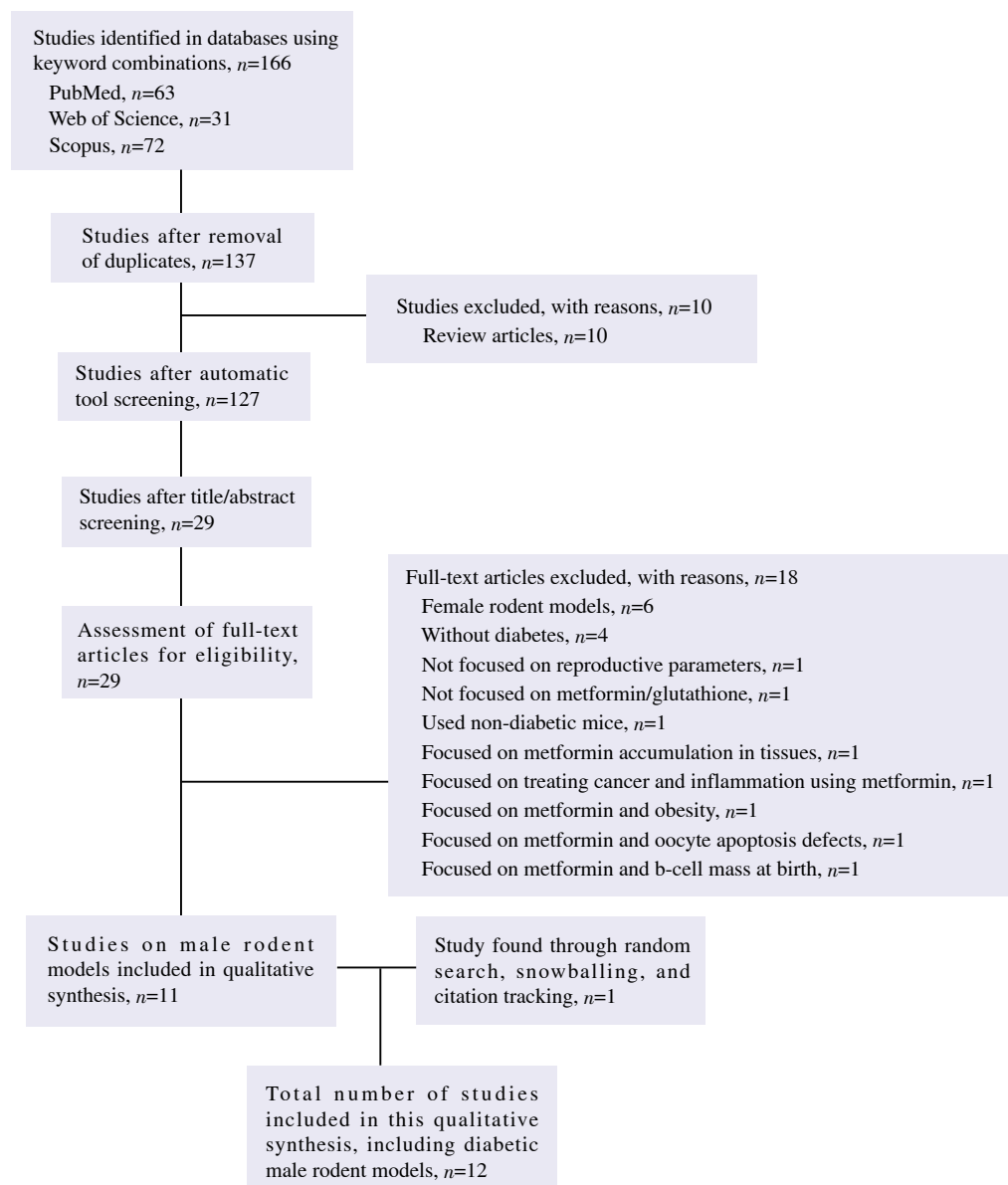


Figure 1. Flowchart of selection of studies for qualitative synthesis.

Table 1. Keywords' combinations used for searching databases.

No.	Keywords combination	PubMed	Scopus	WoS
1	metformin, diabetes, reproduction, glutathione	7	8	4
2	glutathione, diabetes, reproduction, rodent model	21	7	29
3	metformin, diabetes, reproduction, rodent model	35	16	39
4	metformin, diabetes, reproduction, glutathione, rodent model	0	0	0

identified using four combinations of keywords (Table 1) from the PubMed, Scopus, and WoS databases. Automated tools were used to remove duplicate records and review articles, reports, essays, short communications, and mini reviews.

Only eleven studies met the predefined inclusion criteria. However, one additional study was identified through snowballing and citation tracking and was included in the qualitative synthesis. Hence, twelve studies were included in the qualitative synthesis. The results of this review described the effects of administering Met and GSH alone and in coformulation with other compounds on body weight, reproductive organ weight, sperm count, sperm motility, morphology, histopathology, hormone assessment, testicular antioxidants, and OS status in diabetic male rodent models.

3.2. Characteristics of the included studies

The included studies comprised controlled experimental designs using diabetic male rodent models with a control group. Case studies, cross-over designs, studies without separate control groups, non-diabetic disease models, and female animal models

were excluded. All selected studies focused specifically on male reproductive outcomes, including spermatogenesis and sperm quality. Interventions involved the administration of Met and GSH, either alone or in combination with other compounds, across varying doses, duration of administration, and frequencies. The characteristics of the included studies[14,18,23–32], comprising the type of model, sample size, dosage, interventions, and main results, are summarized in Table 2. The effects of Met and GSH interventions on reproductive parameters and male fertility in diabetic rodent models are the focus of the findings.

3.3. Quality assessment of the included studies

The Systematic Review Centre for Laboratory Animal Experiments (SYRCLE) tool was used to determine the "risk of bias" in the included studies (Figure 2)[33]. Each study was assessed independently by RS and FS, and the included studies were categorized based on risk domains (Figure 2). This classification process included subsequent discussions with NAMNK, YSK, and FA to address and resolve any discrepancies in the category.

The majority of research was determined to be at extreme risk of bias due to inadequate description of randomisation (25%, 3/12), allocation concealment (16.67%, 2/12), description of baseline characteristics (8.33%, 1/12), blinding of caregivers/researchers (8.33%, 1/12), selective reporting of results (8.33%, 1/12) and other sources (8.33%, 1/12). Information on animal housing and the randomisation method for outcome assessment was not fully described in any of the included studies, according to the recommendations of the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines[34].

Table 2. Characteristics of the included studies.

Names	Model	Sample	Parameter(s)	Drug/Treatment	Interventions	Results	Conclusion
[14]	Type 2 diabetes mellitus (DM-II) in male Wistar rats	Ninety rats (10-week-old male rats with 160-180 g) were divided into 9 groups (n=10)	Allosteric agonist; testicular steroidogenesis; spermatogenesis	Metformin (Met) and luteinizing hormone / human chorionic gonadotropin receptor (LH/hCG-R)	High-fat diet for 10 weeks followed by a single low dose of streptozotocin (STZ) (25 mg/kg) interperitoneally (<i>i.p.</i>); hCG (15 IU/rat/day) & TP3 (15 mg/kg/day) <i>i.p.</i> in the last five days of Met treatment; 120 mg/kg b.w./day Met (oral) for 5 weeks	In DM-II, hCG weakened the effects of TP3, decreased LH/hCG-R expression after five days, and stimulated steroidogenesis and steroidogenic acute regulatory protein (StAR) gene expression less effectively.	In acute situations, a combination of Met and LH/hCG-R agonists restores testicular steroidogenesis and spermatogenesis in DM-II, but not in chronic conditions.
[18]	Type 1 diabetes mellitus (DM-I) Male C57BL/6NTac mice	Twenty-four mice, (8-12 w/o, 25-30 g), n=6 in each group	Oxidative stress (OS), sperm quality, testicular damage, and seminiferous tubule diameter	Glutathione (GSH)	50 mg/kg b.w. of STZ for 5 days (<i>i.p.</i>) to induce DM-I; 15 mg/kg of GSH weekly for 6 weeks (<i>i.p.</i>); 30 mg/kg GSH weekly for 6 weeks (<i>i.p.</i>)	b.w. reduced by 30 mg/kg in the GSH-treated group; 15 mg/kg GSH reduced sperm motility and seminiferous lumen diameter; Relative testicular weights remained unchanged in treated groups; Antioxidants taken in high doses may cause adverse effects.	Exogenous GSH (15 mg/kg) improved testicular morphology and sperm quality against DM-I.

Table 2. Continued.

Names	Model	Sample	Parameter(s)	Drug/Treatment	Interventions	Results	Conclusion
[23]	DM- I Male adult Sprague-Dawley (SD) rat	Eighteen rats (250-300 g) were divided into 3 groups ($n=6$)	T e s t i c u l a r steroidogenesis, spermatogenesis, a n d m R N A transcript levels	Met	60 mg/kg/day STZ single (<i>i.p.</i>); 300 mg/kg b.w./day Met	Steroidogenesis is disrupted by DM, resulting in abnormal spermatogenesis and decreased fertility.	Met intervention increased sperm parameters, Leydig cell count, testosterone levels, nuclear DNA fragmentation, and steroidogenesis-related mRNA transcript levels.
[24]	DM- II male SD rats	Three-four months old, 250-300 g rats were divided into 4 groups, each group having 8 animals.	D i s o r g a n i z e d germinal epithelium, e d e m a t o u s , thickened interstitial connective tissue in the testis, crowded nuclei, and hormone receptors	Met, Pioglitazone, and Sitagliptin	35 mg/kg STZ (<i>i.p.</i>) ; 100 mg/kg/day Met (oral); 2 0 m g / k g / d a y pioglitazone (oral); 3 0 m g / k g / d a y sitagliptin (oral)	Suppression of estrogen receptor alpha (ER) and estrogen receptor beta (AR β) immunoreactivity in the epididymis and seminal vesicles, as well as a reduction in testicular ER and ER β immunoexpression.	Met has the least negative effects on male reproductive organs in DM- II rats.
[25]	DM- I induced 5-6 w/o male (C57BL/6J) mice with 18-22 g	Groups: Normal control ($n=20$), DM ($n=25$), and DM + Met ($n=25$)	T e s t i c u l a r impairment, apoptosis, Diabetes, and spermatogenic damage	Met	50 mg/kg/day STZ for 5 days (<i>i.p.</i>); 250 mg/kg Met hydrochloride solution (in potable water orally administered for 4 months)	The spermatogenetic cells are morphological indices of spermatogenesis dysfunction, and diabetes causes damage and atrophy of the seminiferous tubules.	Met inhibits testicular injury in diabetic mice by inhibiting the apoptosis of cells.
[26]	Male Wistar rats	Thirty-two rats (250-270 g) were divided into 4 groups with 8 animals in each	B i o c h e m i c a l assessment through blood samples, sperm analysis, histopathology, and morphometric evaluation of the testis and epididymis	Silymarin and Met	60 mg/kg STZ single <i>i.p.</i> ; 200 mg/kg b.w./day metformin; 100 mg/kg b.w./day Met	Met and silymarin treatment improves sperm count, chromatin quality, maturity, motility, viability, sperm DNA, sperm parameters, seminiferous tubule diameter, and testicular histopathological parameters.	Met and silymarin enhance sperm parameters and testicular histopathology; silymarin is more effective than Met in addressing diabetes-induced infertility.
[27]	Diabetic male SD rats	Forty adult rats (250-300 g) maintained in a 12/12 h reversed light/dark cycle were randomly divided into 5 groups ($n=8$)	B l o o d glucose level, weight, and relative weight of reproductive organs, histopathology, and oxidative stress (OS) in testes and epididymis, antioxidant mRNA transcript level, apoptosis, and inflammation-related genes	STZ; Malaysian propolis (MP); Met	60 mg/kg body weight (b.w.) of STZ single dose (<i>i.p.</i>); 300 mg/kg b.w./day MP (oral); 300 mg/kg b.w./day Met (oral)	Co-administration of Met and MP provided the best results in attenuating testicular OS, apoptosis, and inflammation, and may lead to fertility improvement by up-regulating testicular mRNA.	Co-administration of Met with MP provided better results as compared to mono administration of Met in reducing fertility-related diabetic complications in males.
[28]	Male NMRI mice	Seventy mice (1 month old) with 20-25 g, 7 groups with 10 mice in each	DM, OS, testicular tissue changes, seminiferous tubules disruption, Sertoli cell dysfunction	Crocin and Met	600 mg/kg/day methylglyoxal (MGO) for 30 consecutive days (oral) to induce diabetes; 15, 30, and 60 mg/kg/day MGO+crocin for 30 consecutive days (oral); 150 mg/kg/day MGO+Met for 30 consecutive days (oral); 60 mg/kg/day crocin for 30 consecutive days (oral).	Male diabetics who have hyperglycaemia and impaired glucose homeostasis have negative effects on testicular function and spermatogenesis.	Crocin and Met resulted in improved sperm count, testicular volume, and seminiferous tubule morphology.

Table 2. Continued.

Names	Model	Sample	Parameter(s)	Drug/Treatment	Interventions	Results	Conclusion
[29]	DM- I male Swiss albino mice	Eighteen mice, 3 groups with 6 animals in each	D i a b e t e s , vacuolization of seminiferous tubules, testicular steroidogenesis, Leydig cells, plasma leptin level	Met	75 mg/kg/day STZ for 4 days (<i>i.p.</i>); 500 Met mg/kg for 2 weeks (oral)	DM- I affects testicular steroidogenesis and proliferation by reducing leptin levels and leptin receptor (ObR) expression in diabetic mice testes, particularly in the Leydig cells.	In DM- I mice, Met treatment had a positive impact on the testicular steroidogenic pathways, increased testosterone synthesis, and significantly improved testicular histoarchitecture.
[30]	DM- II male Wistar rats	Two-month-old male rats with 170–200 g, 3 groups with 18 animals in each group	Epididymal sperm count, including the spermatozoa with progressive motility, and the percentage of abnormal sperm, Steroidogenesis, spermatogenesis	Met and luteinizing hormone / human chorionic gonadotropin (hCG) receptor (LH/hCG-R)	High-fat diet for 9 weeks followed by a single low dose of STZ (25 mg/kg) (<i>i.p.</i>); hCG (20 IU/rat/day) & TP03 (15 mg/kg/day) <i>i.p.</i> ; 120 mg/kg b.w./day Met (oral) for 4 weeks	In DM- II rats, the Met treatment normalized spermatogenesis and partially restored testosterone levels, increasing the steroidogenic effects of TP03 and hCG on the first day, but not in subsequent days.	Following five days of TP03 and hCG treatment, the percentage of abnormal sperm decreased, and the epididymal sperm count, including progressive motility spermatozoa, was restored in diabetic rats. Met therapy increased testosterone production in diabetic rats on the first day; however, no significant difference was observed in the steroidogenic and spermatogenic effects of LHR agonists post-treatment in diabetic rats.
[31]	DM- I male ICR mice	Five w/o mice, 5 groups with 5 mice in each group	Cellular oxidant products, DM- I, pathogenic effect, glucose metabolism	GSH supplement and insulin	100 mg/kg STZ single <i>i.p.</i> ; 30 mg/kg GSH for 2 weeks intravenous (<i>i.v.</i>); 100 U/kg once a day Insulin, subcutaneous injection	Hyperglycaemia caused the overproduction of ROS in the livers, brains, and kidneys of DM- I mice. GSH significantly suppressed the expression of CuZn-SOD and glutathione peroxidase (GPx) mRNA, while insulin decreased only the expression of GPx mRNA.	GSH supplements have the potential to improve antioxidant balance, leading to a delayed diabetic progression.
[32]	Male Wistar rats	Forty rats were divided into 5 groups (<i>n</i> =8 in each)	P a t h w a y modulation; GSH production; Inflammation	Met and gallic acid	40 mg/kg STZ single <i>i.p.</i> ; Met (100 mg/kg); Met (100 mg/kg) + gallic acid (100 mg/kg)	Co-administration of Met and gallic acid increased the production of GSH, decreased fasting blood glucose levels, alleviated inflammation, and modulated the signalling pathway.	Co-administration of Met performed better than individual treatment of each of Met and gallic acid.

GSH: glutathione; DM: diabetes mellitus; DM- I : type 1 diabetes mellitus; DM- II : type 2 diabetes mellitus; Met: metformin; OS: oxidative stress; ROS: reactive oxygen species; *i.p.*: intraperitoneally; STZ: streptozotocin; b.w.: body weight; LH/hCG-R: luteinizing hormone/human chorionic gonadotropin receptor; TP3: 5-amino-N-tert-butyl-2-(methylsulfanyl)-4-(3-(nicotinamido)phenyl)thieno[2,3-d]pyrimidine-6-carboxamide; TP03: 5-amino-N-tert-butyl-2-(methylsulfanyl)-4-(3-(nicotinamido)phenyl)thieno[2,3-d]pyrimidine-6-carboxamide; SOD: superoxide dismutase.

4. Discussion

The results of this systematic review illustrate the effects of diabetes mellitus (DM- I and DM- II) on reproductive parameters and fertility. The impact of DM on reproductive parameters and fertility, as well as the effects of administering Met and GSH alone and in combination with other compounds on body weight, reproductive parameters, including weight of reproductive organs, sperm count, sperm motility, morphology, and histopathology, hormone assessment, testicular antioxidants, and OS levels, are discussed in detail in the light of existing studies.

Diabetes is a worldwide health concern that primarily affects males with sexual and reproductive issues. The group of metabolic disorders known as DM is characterized by hyperglycaemia, dyslipidemia, and insulin resistance. Diabetes is frequently linked to OS and inflammation because prediabetes' hyperglycaemia and dyslipidemia can weaken the body's overall antioxidant defence[35,36]. Hyperglycaemia-induced OS is crucial for diabetes development and progression, leading to the exploration of antioxidative strategies[36].

Diabetes impairs male reproductive function, leading to a reduction in the normal functioning of various bodily systems

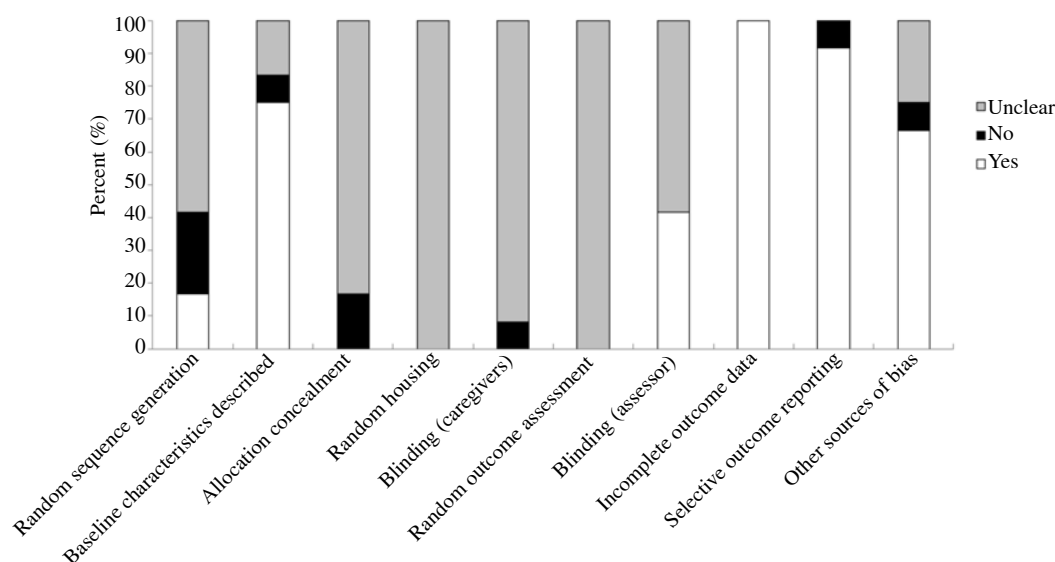


Figure 2. Risk of bias evaluation for animal research using the Systematic Review Centre for Laboratory Animal Experiments (SYRCLE's) risk of bias tool.

due to abnormal energy metabolism and OS[37]. DM significantly reduces testosterone levels, androgen receptor (AR), and luteinizing hormone receptor (LHR) in the testes due to the downregulation of AR and LHR[23]. In addition, DM can cause abnormal sperm motility, testicular alterations, decreased libido, impotence, erectile dysfunction, and hormone abnormalities that result in subfertility and infertility in men[38]. DM can cause a decrease in the diameter of the seminiferous tubules[39]. Testicular dysfunction in diabetes is caused by spermatogenesis, atrophy of the seminiferous tubules, decreased tubular diameter, cell shrinkage, and germ cell death[40]. Consequently, it is crucial to investigate the impact of drugs used to treat DM on reproductive parameters.

Met is a first-line hypoglycemic agent with antioxidant properties and has been shown to reduce OS and improve male fertility[41]. Antioxidant capacity and testosterone levels were also improved by Met, resulting in better semen quality and spermatogenesis in obese mice[24,25]. Met can abrogate not only the decrease in testis weight but also the reduction in the number of spermatogonia, Sertoli cells, and Leydig cells[42]. Met increased sperm count, germinal epithelium thickness, seminiferous tubule diameter, and testicular histology in diabetic rats[26]. Met, with its antioxidant and anti-apoptotic properties, protects the reproductive system and improves sperm count and mobility in diabetic patients with infertility while increasing testosterone hormone levels in blood serum and testicles[43].

Met also improves epididymal epithelial height, decreases Leydig cell number, and decreases testicular tubule diameter[27]. Met has therapeutic potential in treating the diabetic male reproductive system that involves several mechanisms, such as reducing OS and stimulating the adenosine monophosphate (AMP)-activated protein kinase pathway in testicular cells[44]. It lowers blood sugar, cholesterol, malondialdehyde, and triglycerides[28]. Testicular dysfunction occurs to varying degrees in approximately 90% of

male patients with DM. Met was effective in reversing diabetic spermatogenetic cell damage and atrophy of seminiferous tubules, and improved testicular health in DM- I mice[25]. In diabetic male Sprague-Dawley (SD) rats, Met restored testosterone levels and the weight of the reproductive organs (epididymis, testis, and seminal vesicle)[24].

GSH is the immune system's master detoxifier. GSH is a primary endogenous antioxidant produced by cells. The GSH, a tripeptide found in all mammalian tissues, is a crucial criterion for measuring antioxidant levels[45]. Studies have reported OS in β -cells and tissues linked to diabetic complications and a decrease in antioxidant defence. GSH protects cells from excessive ROS, but its metabolites can promote oxidative processes, leading to ROS and free radicals[18]. In diabetes, the GSH/oxidized glutathione (GSSG) ratio decreases, but exogenous GSH administration restores it by scavenging free radicals and reducing OS[46].

GSH deficiency has been shown to result in the instability of the spermatozoa's midpiece, which impairs the spermatozoa's motility and morphology[47]. GSH concentration varies in male reproductive tract tissues, with a significant amount found in testes, gland secretions, and spermatozoa isolated from the epididymis. Intracellularly, GSH is present in spermatozoa and seminal plasma[48]. An animal study found that exogenous GSH improved sperm motility and testicular morphology without affecting blood sugar or body weight, suggesting that exogenous GSH has organ-specific effects without affecting diabetes-related metabolic abnormalities[18]. It has been demonstrated that GSH significantly improves sperm quality[17]. GSH significantly improved sperm quality in infertile men with unilateral varicocele or genital tract inflammation[47]. GSH is a crucial antioxidant for male fertility, protecting sperm membranes and DNA from oxidative damage, preventing reduced motility and increased DNA fragmentation.

Crocin and Met treatment significantly improved sperm count and

testosterone levels[28]. Similarly, in diabetic rats, Met and silymarin treatment improved sperm parameters, sperm DNA integrity, seminiferous tubule diameter, germinal epithelium thickness, and testicular histopathology. The silymarin-treated group showed significant improvements in these parameters[26]. Met decreased leptin receptor (ObR) expression, leptin, testosterone levels, and germ cell proliferation while restoring steroidogenic transport protein expression in DM- I testes[29]. In diabetic rats, pioglitazone and Met together increased antioxidant status[24].

The combination of Met and Malaysian propolis was found to be the most effective anti-hyperglycaemic therapy, resulting in increased weight of epididymis, testis, seminal vesicle, prostate, reduced NF- κ B-mediated inflammation, apoptosis, and epididymal-testicular OS[27]. In DM- II, long-term co-administration of Met and LHR agonists increases the steroidogenic action of 5-amino-N-tert-butyl-2-(methylsulfonyl)-4-(3(nicotinamido)phenyl)thieno[2,3-d]pyrimidine-6 carboxamide (TP03) and gonadotropin[30]. When single-dose LH/hCG-R agonists like human chorionic gonadotropin (hCG) and 5-amino-N-tert-butyl-2-(methylsulfonyl)-4-(3(nicotinamido)phenyl)thieno[2,3-d]-pyrimidine-6-carboxamide (TP3) are treated with Met, their steroidogenic action is markedly increased. This is due to Met's ability to block oestradiol synthesis and aromatase expression in the testes, repair the ultrastructure of seminiferous tubules, and sustain LH/hCG-R expression in testicular Leydig cells[14]. The efficacy of Met on sperm motility may be better than insulin in diabetics[49].

Co-administration of insulin and GSH may delay the progression of diabetes by improving antioxidant balance[31]. GSH administration reduced body and seminal vesicle weight in DM- I mice[18]. Exogenous sources of antioxidants are needed to overcome stress conditions. Nowadays, novel antioxidant therapy is gaining attention due to natural antioxidants' free-radical scavenging capacity and ability to preserve β -cell function by preventing OS-induced apoptosis. The included studies show that Met reduces insulin sensitivity and suppresses diabetes complications, with some novel antioxidant therapeutic approaches. Included studies of GSH were very recent (from 2014 and 2021), demonstrating that the use of GSH in diabetes-induced male infertility is a new idea.

Although the included studies were conducted in rodent models, Met has long been (1950s) used clinically for diabetes and shows benefits in improving testosterone levels and sperm parameters in men[50–52]. Evidence also suggests that GSH supplementation enhances semen quality in human studies[53–55]. The positive findings from rodent models using Met and GSH, their combinations with other compounds, suggest potential therapeutic value for diabetes-related male infertility. These results highlight the need for clinical trials to determine effective dosing, treatment duration, combination strategies, and long-term safety. Overall, both antidiabetic and antioxidant compounds show promising translational potential for improving male reproductive health in diabetic patients.

5. Limitations

This review is limited for several reasons. Firstly, the scope of this systematic literature review is limited to diabetic male rodents, as the fertility and reproductive problems caused by DM in males are alarming. Secondly, we found only two experimental GSH administration articles that met the inclusion criteria in the qualitative analysis. Despite the limited available evidence, GSH may be considered for managing diabetes-induced male infertility. There was not enough evidence to explore the association between Met and GSH when used as adjunctive treatment, which needs to be evaluated in future studies. Another limitation is that only articles whose full text is available in English were included. Despite these limitations, this systematic review provided valuable insights into existing knowledge, research gaps, and possible future research developments.

6. Conclusions and future prospects

The findings of this systematic literature review underscore the potential protective effects of Met and GSH alone and in combination with other compounds to improve sperm parameters, testicular dysfunction, gonadotropin hormones, testicular antioxidants, reproductive health, and fertility. Due to the negative effects of hyperglycaemia and OS associated with DM, there is a growing need for patients to utilise safer antioxidant agents that can alleviate diabetes-related issues. It suggests that using antioxidants (GSH) alongside antidiabetics (Met) may be a promising therapeutic strategy for managing infertility related to type 1 DM, potentially enhancing clinical applications of GSHs. Further studies are imperative to elucidate the role of GSH in reproductive parameters. This will support the development of therapeutic approaches for diabetes, facilitating the translation of emerging therapies into clinical use.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Authors' contributions

Razia Sardar and Fatima Sardar contributed to the conception

and design of the study. Razia Sardar registered the PROSPERO protocol. Razia Sardar and Fatima Sardar thoroughly reviewed the identified studies, including the assessment of titles, abstracts, and full texts. Nor-Ashikin Mohamed Noor Khan, Fathiah Abdullah, and Yuhaniza Shafinie Kamsani reviewed the identification, screening, and selection process conducted by Razia Sardar and Fatima Sardar. Razia Sardar and Fatima Sardar carried out data extraction. The first draft of the manuscript was written by Razia Sardar and Fatima Sardar, and it was reviewed by all other authors (Nor-Ashikin Mohamed Noor Khan, Fathiah Abdullah, and Yuhaniza Shafinie Kamsani). The final paper was read and approved by all authors. The authors resolved their disagreements through discussion.

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