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Francielle de Fatima Viana Santana, Amanda Alves Lozi, Reggiani Vilela Gonçalves, Janaina da Silva, Sergio Luis Pinto da Matta. Comparative effects of finasteride and minoxidil on the male reproductive organs: A systematic review of in vitro and in vivo evidence. *Toxicology and Applied Pharmacology*, 2023, 478, pp.116710-116710. 10.1016/j.taap.2023.116710 . hal-04285258

HAL Id: hal-04285258

<https://hal.science/hal-04285258>

Submitted on 19 Dec 2023

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**Comparative effects of finasteride and minoxidil on the male reproductive organs:
a systematic review of *in vitro* and *in vivo* evidence**

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Abstract

Finasteride and minoxidil are medicaments commonly prescribed for treating benign prostatic hyperplasia (BPA), hypertension, and/or androgenetic alopecia (AGA). The mechanism of action of finasteride is based on the interference in androgenic pathways, which may lead to fertility-related disorders in men. Minoxidil, however, can act in multiple ways, and there is no consensus that its use can adversely affect male fertility. Since finasteride and minoxidil could be risk factors for male fertility, we aimed to compare their impact on the two reproductive organs testis and epididymis of adult murine models, besides testis/epididymis-related cells, and describe the mechanism of action involved. For such, we used the PRISMA guideline. We included 31 original studies from a structured search on PubMed/MEDLINE, Scopus, and Web of Science

databases. For *in vivo* studies, the bias analysis and the quality of the studies were assessed as described by SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation). We concluded that finasteride and minoxidil act as hormone disruptors, causing oxidative stress and morphological changes mainly in the testis. Our results also revealed that finasteride treatment could be more harmful to male reproductive health because it was more associated with reproductive injuries, including damage to the epididymis, erectile dysfunction, decreased libido, and reduced semen volume. Thus, this study contributes to the global understanding of the mechanisms by which medicaments used for alopecia might lead to male reproductive disorders. We hope that our critical analysis expedites clinical research and reduces methodological bias. The registration number on the Prospero platform is CRD42022313347.

Keywords: Male reproductive health; androgenetic alopecia; endocrine disruptors; oxidative stress, morphological alterations

1. Introduction

Finasteride and minoxidil are currently approved by the US Food Drug Administration (FDA) for treating benign prostatic hyperplasia (BPH) and hypertension, respectively. Furthermore, the FDA also recommends the combination of oral finasteride and topic minoxidil for treating male androgenetic alopecia (AGA) [1,2]. Nonetheless, growing evidence supports oral minoxidil use in low doses, which is more efficient [3,4]. In men, AGA reaches about 50% of them being the most common type of alopecia caused by genetic factors associated with an increased rate of conversion of testosterone to dihydrotestosterone (DHT), the most potent natural androgen, a process involving type II 5 α -reductase [5,6]. DHT has been found to be the primary androgen involved in the pathogenesis of AGA by promoting follicular miniaturization [7], as well as, in the pathogenesis of BPH, by preventing the growth of epithelial cells in the prostate [8].

Finasteride is a synthetic anti-androgen that works by competitively inhibiting 5 α -reductase, resulting in the inhibition of the conversion of testosterone to DHT, markedly suppressing serum DHT levels. Testosterone is the predominant androgen in the peripheral circulation, however, conversion to DHT is necessary for biological

activity in some organs, such as liver, skin, seminal vesicles, and prostate [9]. Although this drug has been proven effective at reducing male pattern hair loss though the reduction of DHT levels, there has been debate over the adverse sexual side effects it could produce. Since testosterone production is controlled by a negative feedback loop [10], this medication could affect steroidogenic pathways in Leydig cells (LCs) or within the hypothalamic-pituitary-gonadal (HPG) axis by increasing the serum testosterone levels. Furthermore, histomorphological and functional damage in the dependent tissues of a normal rate of testosterone could lead to some adverse effects during the treatment. Studies have proved that finasteride impairs male fertility [11,12,13]. The adverse effects on male fertility commonly related to this drug include decreased libido, erectile dysfunction, reduced ejaculatory volume, and temporary reduction in sperm count, which is caused by the imbalance of testosterone conversion [14,15,16].

Although minoxidil is also used to treat AGA, its mechanism of action is not well known. The process should occur through multiple pathways (vasodilatation, inflammatory action, and induction of the Wnt/b-catenin signaling pathway), including antiandrogen pathways [17]. In this partially unknown scenario, some authors point out that the hair-stimulating effect of minoxidil is not mediated by an antiandrogenic mechanism, such as finasteride since significant changes were not found in the testosterone levels in their experiments [18,19]. However, *in vitro* findings suggest that minoxidil increases the activity of enzymes from androgenic pathways, such as 17 β -hydroxysteroid dehydrogenase (17 β -HSD), involved in the last step of the testosterone synthesis, [19] and 5 α -reductase enzyme [20], which could impair male fertility. Nonetheless, the literature regarding the minoxidil effects on male reproductive health is scarce despite the contemporary significance of this subject.

Wu et al. (2016) [21], who focused on understanding alopecia considering the side effects of minoxidil and finasteride for both women and men, assessed the FDA Adverse Event Reporting System (FAERS), a computerized information database established by the U.S. Government, which restores reports of adverse events for drug products approved by FDA. The authors retrieved events related to finasteride or minoxidil, from January 2004 to June 2014 and confirmed that finasteride was more associated with reproductive injuries by patients (sexual disorder, erectile dysfunction, decreased libido, and reduced semen volume) compared to minoxidil [21]. However, human studies that carried out an analysis of morphological, molecular, biochemical, or hormonal comparison of the effects of these two approved drugs on male reproductive organs have not yet been developed.

In this context, a systematic review of preclinical studies, *in vitro* and *in vivo*, is essential to understanding the main cellular pathways activated after finasteride and minoxidil exposure mainly in the testis and epididymis, since no study compared these two drug effects in these organs. Therefore, our results may provide new insights into male repro-toxicology, male reproductive health, and therapeutic alternatives for the treatment of alopecia. Thus, this study contributes to the global understanding of the mechanisms induced by medicaments used for alopecia, which might lead to male reproductive disorders.

2. Methods

2.1 Focus question

The main question to be answered in this systematic review was: What is the impact of finasteride and minoxidil on the testis and epididymis based on preclinical model (*in vivo* and *in vitro*) evidence?

2.2 Search strategy

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [22]. Further information about the protocol for this systematic review was registered on PROSPERO - International Prospective Register of Systematic Reviews (CRD42022313347). Details about the Population, Interventions, Comparators, Outcome, and Study design (PICOS) are given in Table S1. A bibliographic search was performed using a two-step search: (1) direct search in electronic databases PubMed/MEDLINE (<https://www.ncbi.nlm.nih.gov/pubmed>), Scopus (<https://www.scopus.com/home.uri>), and Web of Science (<https://www-periodicos-capes-gov-br.ez35.periodicos.capes.gov.br>) completed on August 26th, 2021, at 08:43 am, and (2) indirect screening of reference lists from all the included studies identified in the direct search [23]. The keywords used as search filters were organized into four groups: animal models, organ (testis and epididymis), finasteride, and minoxidil, which were combined by Boolean connectors [AND] among them or [OR] between the two drugs. Search filters were initially created for the PubMed/MEDLINE database, using algorithms [MeSH Terms], which means indexed records, and [TIAB], which refers to papers recently published in an indexing process [24]. The same research descriptors were structured according to specific search algorithms required in Scopus and Web of Science databases. The search strategy is detailed in the supplementary materials (Table S2).

2.3 Eligibility Criteria

After the record identification through the databases, removal of duplicates, and initial screening, the full text of potentially relevant studies was retrieved and assessed by two independent researchers for eligibility for inclusion in the review.

Only studies that met the following eligibility criteria were selected: (i) *in vivo* studies that analyzed the finasteride or the minoxidil exposure effect on the testis and epididymis of adult murine models; (ii) *in vitro* studies that analyzed the finasteride or the minoxidil exposure effect on the testis/epididymis-related cells.

Study exclusion was based on well-defined criteria, as follows: (i) Studies that used other organs/cell lines or evaluated female animals or other animal models, (ii) Studies containing exclusively non-relevant aspects for our systematic review, such as the exploration of the chemical structure of the enzyme 5α -reductase or the pharmacological profile of drugs, (iii) Studies that administrated only finasteride or minoxidil as a positive control, (iv) Studies lacking drug exposure, (v) Studies of behavior, (vi) Studies with treatment association, (vii) Secondary studies as reviews and (viii) Studies in a language other than English (ix) Studies *ex vivo*. The reference list of each included study was manually checked to identify additional studies. If a record was identified in this step, its reference list was also revised.

The kappa test was used for selection and data extraction (kappa= 0.870). Two reviewers (FFVS and AAL) independently conducted the literature search, removed duplicate articles, and examined titles and abstracts according to eligibility criteria. In case of discrepancies, a third reviewer (JS) decided whether the study met the inclusion criteria.

2.4 Data extraction and synthesis from *in vivo* and *in vitro* studies

Considering all included studies, data extraction according to our objectives was based on descriptive levels, as follows: (i) characteristics of publication: authors,

publication year, and country; (ii) characteristics of the experimental animals or testis/epididymis-related cells: animal model, age, weight, number of animals, number of animals per group and number of groups; or the cellular type, cell lineage source, and culture medium; (iii) Intervention: drug, drug vehicle, drug dose, timing of exposure, route, and control compounds, (iv) comparison of primary outcomes and molecular pathways activated after finasteride and minoxidil exposure in preclinical models

2.5 Risk of bias assessment from *in vivo* studies

The quality of the included animal intervention studies was assessed using the criteria described in the risk of bias (RoB) tool of SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) [25]. The following types of bias described by the RoB tool were evaluated: selection bias (sequence generation, baseline characteristics, allocation concealment); performance bias (random housing and blinding of caregivers or investigators); detection bias (outcome assessment, blinding of outcome assessment); attrition bias (incomplete outcome data); reporting bias (selective outcome reporting); and other sources of bias (the presence of ethics committee, which treatment the control group received and the omission of the route of administration). The items in the RoB tool were scored with “yes” (low risk of bias), “no” (high risk of bias), or “unclear” (indicating that the item was not adequately reported and, therefore, the risk of bias was unknown) [24]. The results of the risk of bias across the included studies were graphically expressed using the Review Manager 5.3 program from Cochrane Collaboration (RoB 2.0).

3. Results

3.1 Included Studies and Characteristics of Publication

Our search strategy allowed for recovering 1367 records (PubMed/MEDLINE: 190, Scopus: 577, and Web of Science: 600). After removing 524 duplicates, 843 papers were screened by reading the title and abstract. Thus, 763 studies were excluded due to the absence of the established criteria (eligibility criteria). Eighty studies were assessed through the eligibility criteria, then 49 articles were excluded. Therefore, 31 records [26-56] were included in this systematic review. Out of these, 27 studies (24 *in vivo* and 3 *in vitro*) analyzed the finasteride effects and four studies (3 *in vivo* and 1 *in vitro*) examined the minoxidil effects on male reproductive organs (Fig. 1). No recovered study compared the two drugs.

The supplementary material contains the main characteristics of publication and experimental animal models or cell culture. The studies that administrated finasteride *in vivo* were published from 1991 to 2020 and were conducted in several countries, mainly the United States of America and Poland (25% each), followed by Brazil (12.5%), China, and Iraq (8.33% each). The three *in vitro* studies that investigated the effect of finasteride were published from 1993 to 1998 and were mainly conducted in the United States of America (66.66%). The three studies that administrated minoxidil *in vivo* were published from 2002 to 2020 and were from China, United Kingdom, and Turkey. The *in vitro* study that investigated the effect of minoxidil was published in 1982, and the country where the assay was performed was not informed.

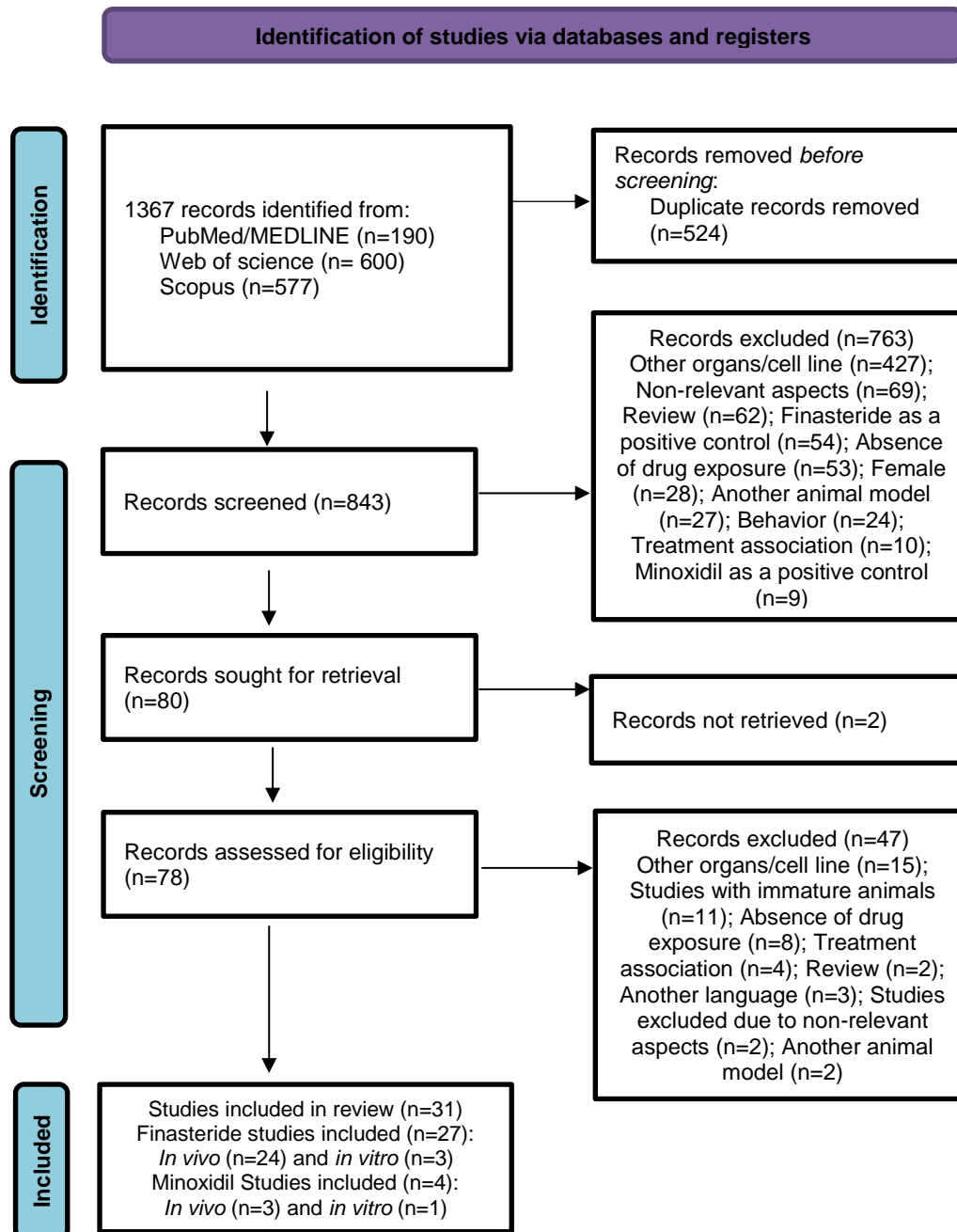


Fig 1. Flow diagram of the systematic review literature search results based on PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) (<http://www.prisma-statement.org>).

3.2 Preclinical studies: Characteristics of Experimental Design

Considering the animal models, the 24 studies that administrated finasteride *in vivo* mainly used rats (83.33%) [26,28-35,37-39,41-46,48,49], mostly Sprague Dawley and Wistar rats. The other finasteride studies used mice (12.5%) [27,36,40], such as Balb/c strains, except one study that used a wild rodent, the *Mesocricetus auratus* [47], as the he animal model. Regarding the three *in vivo* minoxidil studies, Wistar rats were the most used (66.66%) [51,52]. One study used KM mice [50] (Table S3).

The main experimental characteristics of the studies are described in Table S4. Concerning the finasteride exposure, almost 37.5% of the records did not inform how the drug was solubilized [31-34,36,44,46,47,49], while 20.83% of the studies dissolved it in methylcellulose [28,29,38,40,48], followed by 16.7% in water [27,30,37,39]. The lowest finasteride dose used was 0.01 mg/kg [30], and the highest dose was 250 mg/kg [40], both daily, by oral route. However, the vast finasteride doses used were 5 mg/kg (37.5% of the studies)[29,31-34,37,42,44,46], 20 mg/kg (12.5%)[35,46,48], and 1.5 [36,38], 10 [37,38], 25 [27,38], or 80 mg/kg [28,48] (8.33% each). The experiments that administrated finasteride lasted from 5 days to 10 months. Most studies assessed the effect of the drug administered daily (91.66%) [26-40,41-46,48,49], but two others offered only 3 [47] or 5 times/week [41] (4.16% each). The treatment was mainly supplied by oral route (75%) [27-34,37,39-44,46,48,49] , followed by subcutaneous [35,47] (12.5%) and intraperitoneal routes [38](4.16%). Regarding the control group, half of the studies with finasteride administered the vehicle for the control animals. However, some studies (12.5%) determined not to refrain from administering anything [42,43,49]. Around 33% of the studies did not specify if the animals received something as a placebo [31-34,36,44,46,47].

Concerning minoxidil, it was administered in a pure form [50, 51] or dissolved in sterile water [52]. The minoxidil doses varied from 0.3 mg/kg [51], 15 mg/kg [52], and 20 mg [50]. The experiments lasted from 30 seconds to 21 days. Two studies assessed the drug's effect as a single dose [51, 52], and one study administered the drug once a day for 21 days [50]. The minoxidil

exposure was carried out by oral route (n=1) [52] or intravenously (n=1) [51]. One study did not mention the route used [50]. About the control group, most studies (n=2) administered the vehicle for the control animals [50, 52], and only one study did not administer anything [51].

Regarding the preclinical studies in *in vitro* models, as reported in Table S5, Leydig tumor cells (n=1) [53], the Sertoli cells[54], and cells of testicular parenchyma (n=1)[55] extracted from Crl:CD BR rats, were selected by the included studies that assessed the finasteride impact. No study used epididymis-derived cells. Regarding the culture medium, one study used Waymouth's MB 752/1[53], and one used the RPMI-1640 medium [55]. Only one study did not inform the culture medium used [54]. The only recovered *in vitro* study that investigated the effect of minoxidil used rat testis cells from Sprague-Dawley rats [56]. The culture medium was not described.

As reported in Table S6, among the three *in vitro* studies that assessed the finasteride effect, one reported to have administered finasteride + 1 nM Dibutyryl-cAMP dissolved in ethanol 200-fold concentrated stock or, in the second assay, finasteride + 10 µg/ml 22OH Cholesterol [53]. The second study combined finasteride with conjugate T (1 mg/l) or T: BSA (10 mg/l), and, in the second assay, finasteride with DHT (1 mg/l) or saline solution [54]. The other study added human chorionic gonadotropin (hCG) (500 IU/ml stock solution) or 10 ml of distilled water in the finasteride solution [55]. The finasteride dose used by these *in vitro* studies ranged from 0.0001 to 1 mg/l, and the incubation period ranged from 20 min to 3 h at a temperature from 22 °C to 37 °C. The study that assessed the minoxidil effect administered this drug alone or in the minoxidil + hCG (0.1 or 10 mU) formulation, both in a medication concentration of 0.33 mg/l, 3.33 mg/l, and 33.3 mg/l. The incubation period was 90 min at 37 °C [56].

3.3 Main outcomes: Comparison of the molecular pathways activated after finasteride and minoxidil exposure in preclinical models.

Through our search strategy, which had the same sensitivity for recovering studies that analyzed the effects of finasteride and minoxidil on male reproduction, we retrieved six times

more records *in vivo* that examined finasteride than minoxidil. Despite the limited number of studies evaluating the minoxidil exposure effects, it was possible to compare the histomorphological, molecular, and hormonal effects of both drugs especially in testicular tissue. No recovered study with minoxidil considered epididymis in their evaluation, hence there is no possibility of comparison. *In vitro*, the possibility of comparison is even more limited than *in vivo* due to the reduced number of studies for both drugs. Firstly, only some studies in this field used testis-related cells, and no recovered study used epididymis-related cells. Furthermore, the finasteride and minoxidil included studies *in vitro* have only in common their hormonal level investigation. Therefore, the comparison between the two drugs was limited to hormonal aspects.

Main hormonal outcomes

Thirteen studies that analyzed finasteride action included in their investigation serum hormonal analyses (Fig. 2- left yellow box), 85% of which observed some hormonal alteration, mainly decreased serum DHT levels (7/8 studies) [29,32,33,38,42,43,45]. However, no study evaluates the DHT levels after minoxidil treatment. Decreased DHT levels after finasteride treatment are expected since finasteride is a 5α -reductase enzyme inhibitor. So far, it has been reported that the lack of this androgen could impact the adult testis, mainly the typical composition of the epididymal fluid crucial for sperm functionality. Three studies [28,39,48] extended their investigations to the effects of finasteride on sperm quality and fecundity (Fig. 2- green boxes). In this approach, some studies revealed that, after finasteride exposure, the animals presented poor sperm quality, including decreased number, morphology, motility, and live spermatozoa associated with low DHT levels.

Finasteride records found increased serum testosterone levels (3/10 studies) [27,29,43]. However, 4/10 studies assessing the finasteride effect *in vivo* also identified a decrease of testosterone [30,32,33,42]. Also, some studies found a decrease in the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [27,30] levels (Fig. 2 left yellow box). Regarding minoxidil, two studies *in vivo* [50, 52] reported that the treatment caused hormonal alterations, including decreased serum testosterone levels or decreased testosterone production in testis (Fig.

3, left yellow box). This result may be included in the mechanism of action of both drugs or even indicate lesions in LCs or interference with the HPG axis.

In the testis-related cells, one study *in vitro* [55] assessing finasteride's effect found a decrease in testosterone and estradiol (E2) levels (Fig. 2, right yellow box). The cells were treated with a very low finasteride dose (0.001, 0.01, and 0.1 mg/l). In the same way, there was a decrease in testosterone production *in vitro* when minoxidil was administered at doses of 0.33, 3.33 and 33.3 mg/l [56] (Fig. 3, right yellow box). An *in vitro* study with finasteride [53] strongly suggest that finasteride can decrease testosterone production by directly affecting LCs. The study demonstrated decreased activity of the side-chain cleavage of cholesterol enzyme involved in transforming pregnenolone into steroid hormones, such as testosterone or estrogen. However, although minoxidil treatment also led to decreased testicular and serum testosterone levels, it is unknown whether this drug affects LCs since no minoxidil study evaluated exclusively this cellular type.

Main histomorphological outcomes in the testis

The purple boxes in Figures 2 and 3 show the histomorphological alterations caused by the finasteride and minoxidil treatments, respectively. The histomorphological results revealed that both drug treatments cause detached germ cells, vacuolization, and degeneration of the seminiferous tubules, which are testis cell death signs (finasteride [27,31,33,34,37,40,41,42,43,47], minoxidil [51]). Most of finasteride studies indicated that finasteride might act as an endocrine disruptor and affect the synthesis and/or distribution of hormones and hormone deficiency leads to changes in morphology by programmed cell death exemplified by confirmed apoptosis and necrosis [27,37,43]. Although the exact mechanism cannot be elucidated for minoxidil, hormonal findings and also the histomorphological changes included increased noncohesive germ cells and packed seminiferous tubules give the first insights that after the administration of the drug, the lack of hormones lead to damage in the tissue morphology or cell death.

Our results show that finasteride treatment also affects the blood-testis barrier (BTB) proteins characterized by weakly immunostaining of N-cadherin and β catenin, and occludin [34] (Fig. 2, right blue box). Furthermore, our results showed increased vinculin tight junction protein immunostaining in germs cells in different stages of the seminiferous epithelium cycle [34], probably altering the BTB. The BTB function is stimulated by testosterone, thus decreased testosterone levels after both drug exposure events visualized in this review may adversely affect the integrity of BTB.

Reactive and nitrosative oxygen species in testis

Comparing the biochemical results (left pink boxes Fig. 2 and 3), two studies [39,42] that exposed animals to finasteride and one [51] that investigated minoxidil visualized the occurrence of oxidative stress in the tissue, especially by the generation of the reactive oxygen species (ROS) and/or nitrosative oxygen species (RNS) and the increase of oxidative stress markers expression like malondialdehyde (MDA).

The decrease of antioxidant enzyme activity (such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPx)) leads to an increase in the free radicals, especially peroxynitrite (ONOO^{*-}) and hydroxyl radical (OH^*), which lead to lipid peroxidation and reflected in the high levels of MDA characterizing the cell degeneration and can progress to cell death. Morphological testis cell death signs were visualized after both drugs administration. In addition, apoptosis was confirmed by one study using finasteride [27] that identified changes in genes and protein expression that led to apoptosis (Fig. 2, left bottom pink box). It was observed an increase in *p53*, *Casp3*, and *Bax* (pro-apoptotic marker) expressions and a decrease in *Bcl2* (anti-apoptotic marker) expression in spermatogenic cells. These alterations may be related to increased MDA levels. Indeed, one study that accessed the effect of finasteride on the testis [39] visualized an increase in MDA levels associated with reduction in the number, morphology, and motility of the spermatozoa. Furthermore, one Minoxidil study [51] also confirmed the association of high MDA levels and histomorphological alteration. However,

although individual studies show that minoxidil hurts cells, in our review was not possible to conclude whether minoxidil could lead to apoptosis or decreased sperm quality, as observed after finasteride use, especially because of the high heterogeneity among the individual studies.

In addition, nitrosative stress could be another cause of BTB interruption. A study with finasteride [33] observed in the cytoplasm of germ cells and Sertoli cells (SC) (both components of the BTB) in different stages of the seminiferous epithelium cycle the immunoreaction for iNOS isoform (Fig. 2, top right blue boxes) that participates in the formation of RNS, which in testis is possibly associated with cell death or interferes with the morphology of the tight junction between SCs. Once the SC is damaged, many histomorphological changes can be visualized because of its intrinsic relationship with germ cells. Nevertheless, a possible rupture in BTB after finasteride exposure could be caused by SC damage mediated by testosterone or nitrosative stress. We cannot conclude that minoxidil has the same mechanism as finasteride since there is not analyzes that identify this marker.

Imbalance redox in mitochondria and endoplasmic reticulum (ER)

Another interesting mechanism described by the individual studies included in this review was the cross-talk between mitochondrial oxidative stress and endoplasmatic reticulum stress (ERS) after the exposure. In our review, ERS and apoptosis markers, such as GRP-78, p-IRE1, and p-JNK54, were found in the testis when finasteride was administrated [43] (left bottom pink box Fig.2), showing the direct relationship between the activation of these organelles during finasteride exposure, especially by stimulating the release of calcium out of the endoplasmatic reticulum. This mechanism could cause cellular damage after drug exposure, and it could be relevant for further studies.

Main effects of finasteride in the epididymis

Although 13 studies examined finasteride effects on the epididymis, no recovered study with minoxidil considered this organ in their evaluations. Nine of these 13 studies found some epididymal alteration caused by the drug administration as biometric changes (Fig. 2 epididymis brown box). Through immunohistochemical analysis in the epididymis, some studies [32, 33]

recognized differences in protein expressions after finasteride treatment as SOD and iNOS, the proteins described early related to ROS formation (Fig. 2 epididymis blue box). The finasteride molecular impact on epididymis was verified for only one included study, which found decreased glutathione peroxidase 5 (GPX5) transcript and increased extracellular superoxide dismutase (E-SOD) transcript in the caput epididymis region, both transcripts of antioxidant proteins involved in tissue defense against free radicals [32] (Fig. 2 epididymis pink box).

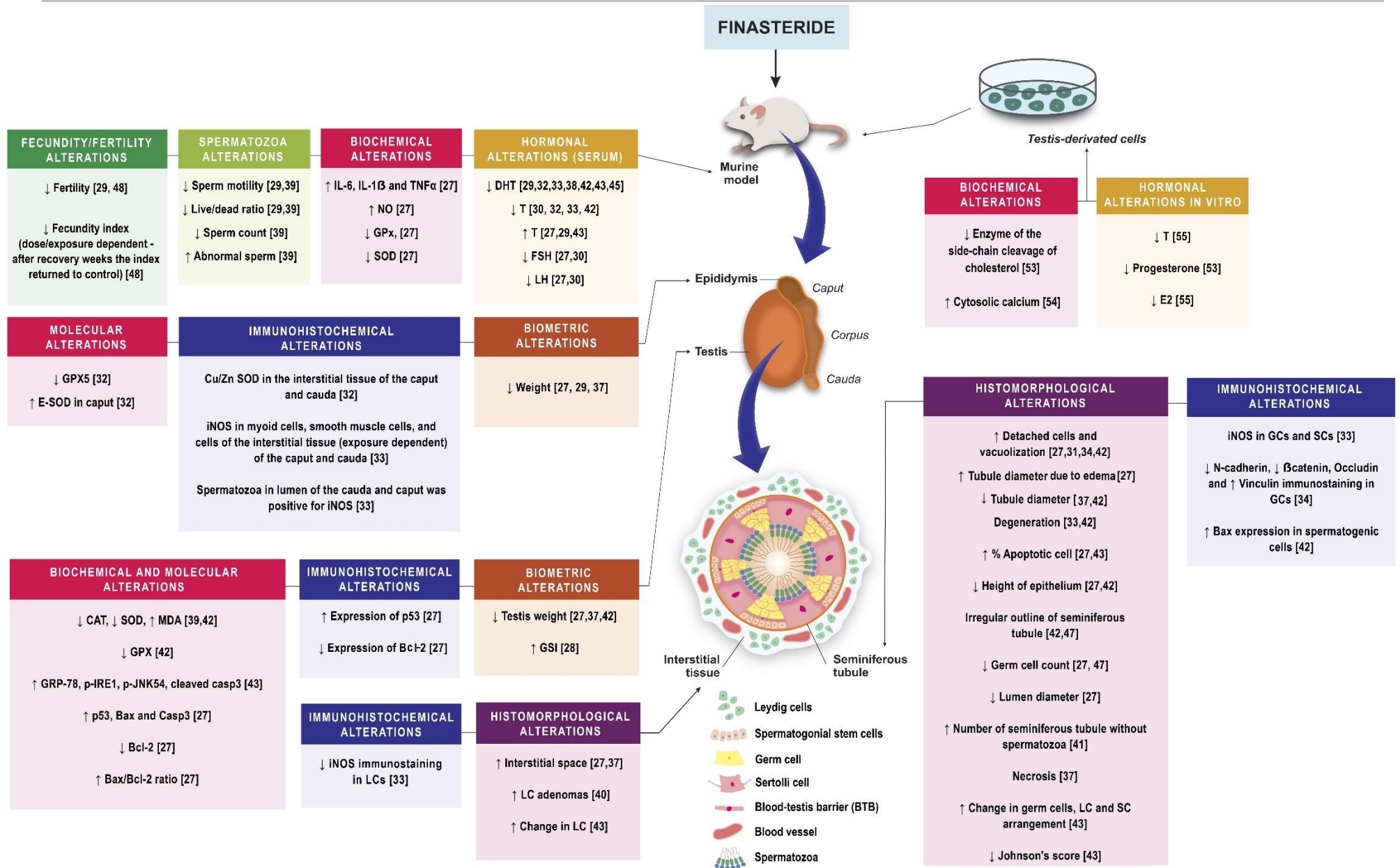


Fig. 2. Main effects of *in vivo* and *in vitro* finasteride treatment on the testis and epididymis of murine models. ↑ = upregulation/increase; ↓ = downregulation/decrease; Bax = Bcl2-associated X protein; Bcl2 = B cell leukemia/lymphoma 2 ; CAT = catalase; Casp3 = Caspase-3; DHT = dihydrotestosterone; E2 = estradiol; E-SOD = extracellular superoxide dismutase; FSH = follicle stimulating hormone; GSI = gonadossomatic index; GPx =

glutathione peroxidase ; GPX5 = glutathione peroxidase 5; IL-6= Interleukin 6; IL-1 β = Interleukin 1 beta; iNOS = inducible nitric oxide; LC = Leydig cells; LH = Luteinizing hormone; MDA = malondialdehyde; NO=Nitric oxide; p53= Tumor protein P53; SC = Sertoli cells; SOD = superoxide dismutase; T = testosterone; TNF α = Tumour Necrosis Factor alpha.

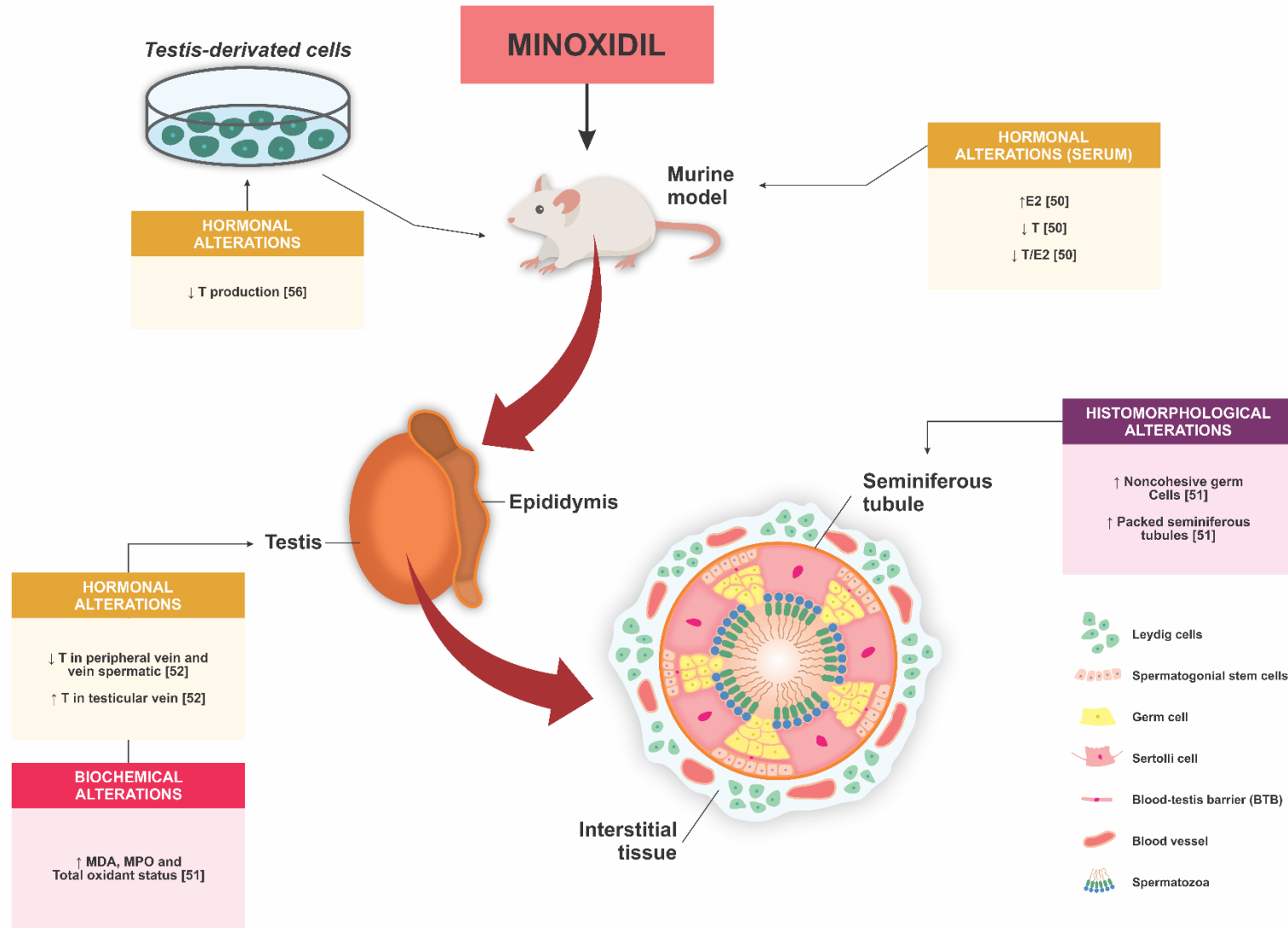


Fig. 3. Main effects of *in vivo* and *in vitro* minoxidil treatment in the testis of murine models. \uparrow = increase; \downarrow = decrease; E2= estradiol; T = testosterone; MDA = malondialdehyde; MPO = Myeloperoxidase.

3.4 Bias report from *in vivo* studies

The bias analysis based on the SYRCLE's tool is presented in Fig. 4a and 4b, which show the percentage of each risk of bias item across the included studies that assessed finasteride or minoxidil. The risk of bias analysis of individual studies is available in Supplemental files – S7. None of the studies fully met all the established criteria for any drugs. For those studies that administrated finasteride (n=24), none of the studies reported random sequence generation or random outcome assessment, which sets a high risk of bias. Regarding the blinding of participants and personnel (caregivers and investigators) and blinding of outcome assessment, just one study suggests that there was blinding (unclear risk of bias), while the others did not give this information and set a high risk of bias. Around 95.83% of the studies followed the methodology and presented the proposed results (low risk of bias), while 4.16% did not describe any methodology or have just informed that it was performed, which sets an unclear risk of bias.

Among the three studies that administrated minoxidil, allocation concealment, random sequence generation, blinding of participants and personnel (caregivers and investigators), blinding of outcome assessment, and random outcome assessment reveal a high risk of bias. All studies followed the methodology and presented the proposed results, thus presenting a low risk for reporting bias.

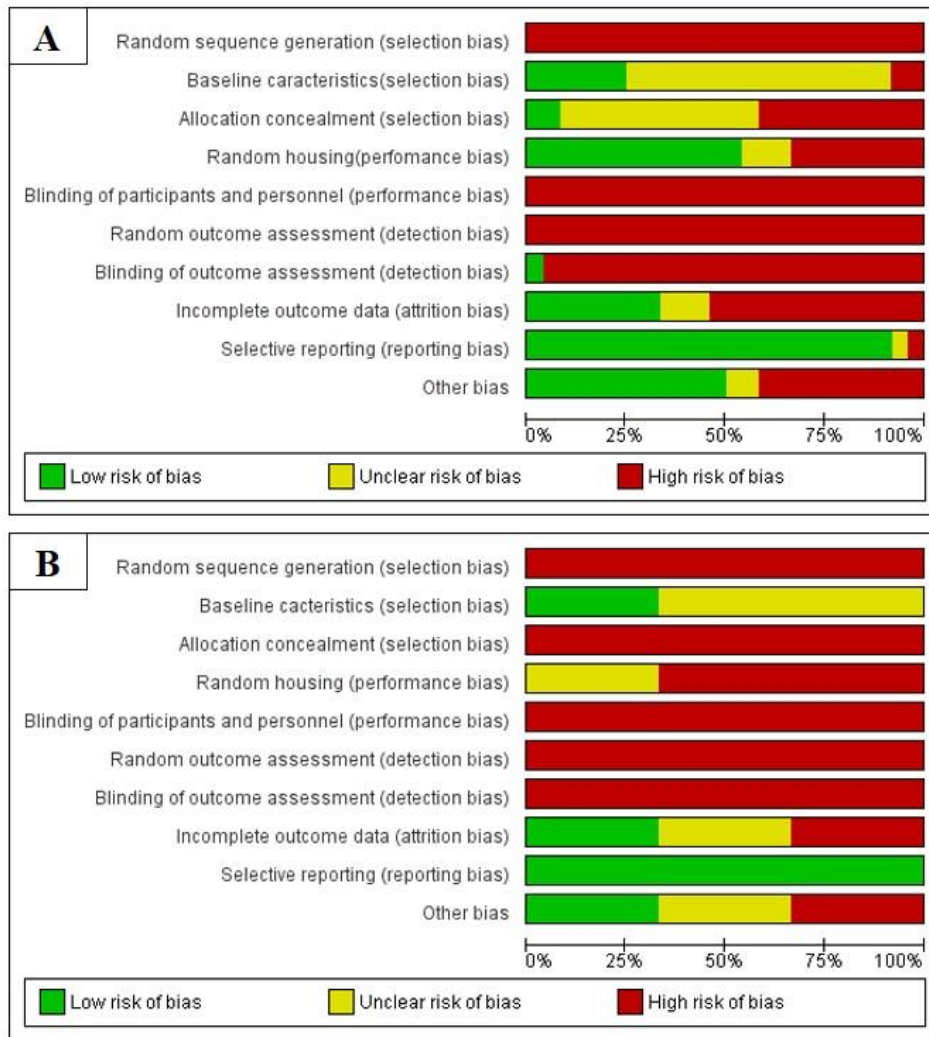


Fig. 4. Results of the risk of bias and methodological quality indicators for all included studies that evaluated the A- Finasteride and B- Minoxidil impact on testis and epididymis of murine models. The items of SYRCLE's RoB tool were scored with "yes" (low risk of bias), "no" (high risk of bias); or "unclear" (indicating that the item was not adequately reported and, therefore, the risk of bias was unknown).

4. Discussion

In our systematic review, we have assessed and compared for the first time the impact of two important medicaments, finasteride and minoxidil, both used to treat alopecia, on male reproduction. Two reproductive organs of adult murine models, testis and epididymis, in addition to testis/epididymis-related cells, were focused on this investigation. Our results demonstrate that both drugs can harm murine models' testis

and testis-derived cell cultures. Furthermore, our outcomes demonstrated that finasteride could damage the epididymis and decrease sperm quality. However, due to the lack of studies on minoxidil's effects on epididymis, it is impossible to determine if this drug affects this organ. Our results also reveal that both drugs could act through mechanisms involving hormonal disruptions and oxidative stress, which will lead to morphological changes that prevent the testis from functioning normally and result in poor sperm quality and decreased fertility.

In this review, the studies that assess the effect of the drugs *in vivo* were performed mainly in the United States of America (1991-1998) and Poland (2004-2011) for finasteride; and China (2020), the United Kingdom (2002), and Turkey (2020) for minoxidil. Two out of three *in vitro* studies that investigated the effect of finasteride were conducted in the United States of America (1993 and 1998), and regarding the only study that evaluated minoxidil, the country where the assay was performed was not informed. These two medications were tested and approved by the FDA for the treatment of AGA in 1997 [1,2] and more recently (2016) by the European Medicines Agency (EMA) [57, 58]. In this period, although both drugs were already available for treating other conditions, they became widely used. Thus, this explains the number of preclinical studies performed in the USA and Poland before or at this period. Since the population has been widely exposed to the medication over the past years, reports to health agencies about the possible adverse effects of use are more consistent, and preclinical and clinical research must be performed to find new approaches to the therapy. In this way, new studies dated in mid-2020 have emerged in other continents, as exemplified by the studies included for minoxidil.

We recovered more articles that evaluated the effect of finasteride than minoxidil on the chosen organs and related cells. However, our search strategy had the

same sensibility for recovering both studies. It is known that finasteride acts by inhibiting the transformation of testosterone into DHT [59]. Therefore, due to its effect on the androgenic metabolism, testis, the organ responsible for spermatogenesis and male sex hormone production [60], and epididymis, which plays a crucial role in spermatozoa maturation [61] have become the targets of a considerable number of studies on the adverse effects of finasteride. On the other hand, the FDA approved minoxidil in 1970 [62] for treating severe hypertension, a systemic disease unrelated to infertility [63]. Besides, since minoxidil is often used in combination with other medications, such as diuretics [64], and it may be challenging to relate secondary effects exclusively to this drug. In addition, some studies have demonstrated that although both drugs were effective and safe in treating mild to severe AGA, treatment with oral finasteride was more common among this group by its efficiency, although related to more side effects [21].

Regarding the duration of the experiment that evaluated finasteride, 56 days was the most used, although several experiments varied from 5 days to 10 months. The information about the exposure time is crucial because it has already been shown that the duration of the cycle of the seminiferous epithelium is 56 days in rats [60] and that, on average, ten days of life for a rat in the fertile phase is equivalent to one human year [65] therefore is more than enough time to study the adverse effects in the stages of the cycle and also in the more prolonged exposure visualizing the impact of chronic exposure. Surprisingly, the studies that assessed the effect of minoxidil on the testis lasted only 30 seconds, 4 hours, or 21 days, aiming to evaluate the acute effect of the substance. The finasteride studies included in this review chose the oral route mainly because therapeutic agents are usually administrated this way to achieve systemic results. However, the minoxidil studies were divided into oral and intravenously

injections. The most used doses were 5 or 20 mg/kg of finasteride. Doses of up to 80 mg/day in humans have been tolerated for treating BPH [66]; however, for AGA, the amount of 1 mg/day has already shown results for treating the condition [67]. Thus, the variation in doses can be essential to verify whether the adverse effects are dose-dependent. In the studies included here, the amount of minoxidil used were 0.3, 15, and 20 mg/kg. The authors did not clarify the reason for choosing these doses. Minoxidil is generally used topically, 1 mL of 2% solution formulation is applied twice daily in humans. However, more recently, doses ranging from 0.5 to 5 mg have been used orally in treating AGA [68], which justifies the administration chosen by one included study.

In our review, we observed that the majority of the included studies employed rats as their chosen experimental murine models, followed by mice. The rat is the animal model more characterized in the toxicology, developmental and reproductive physiology, and endocrinology research fields, mainly due to its sensitive response to interventions [69]. In this review, we also observed that some *in vitro* studies relied on rat-derived testis cells. Stocks (2004) [69] highlighted that many of the proposed *in vitro* tests require animal tissues or receptors derived from animal tissues. However, many authors emphasize that while *in vitro* models have certain limitations, such as absorption, metabolism, distribution, and excretion *in vitro* tests remain valuable because they allow the exclusion of side effects.

Although the studies included in this review describe the drop in testosterone and some consequences to the testicular tissue after finasteride administration, these studies did not clarify the mechanism involved. The HPG axis plays a crucial role in regulating fertility by synthesizing and secreting hormones [70]. The recovered results revealed testosterone reduction, changes LH and FSH levels after finasteride exposure, which, all together, indicate that the HPG axis could be disrupted after finasteride use.

Regarding minoxidil, to the best of our knowledge, there is no evidence that this drug can affect the HPG axis. Hormone deficiency, as consequence of the disruption of HPG axis, can lead to alterations in reproductive organs, such as morphological alterations resulting in cell death, as confirmed by our findings.

According to our outcomes, another remarkable histomorphological damage is the impact on BTB, which possibly occurs through two distinct pathways i.e. mediated by hormones or by imbalance redox. It is already known that BTB plays a vital role in normal testis morphology as an immunological barrier to segregate the postmeiotic germ-cell antigens from the systemic circulation. It creates a unique microenvironment (called adluminal compartment) for germ-cell development and confers cell polarity, essential to spermatogenesis [71]. It is formed by proteins found in adherent or tight junctions (i.e., α -actinin, fimbrin, vinculin, spin, gelsolin, and zonula occludens-1), which have their expression modulated by testosterone [72]. Also, this hormone has been shown to control the cytoplasmic transit by stimulating protein related to endocytic pathways, such as clathrin or proteins involving in transcytosis (caveolin-1 and Rab11, for example) [73]. Therefore, the lack of the hormone allows the permeability of the BTB to toxicants, which leads to disorganization of the adluminal compartment. Thus, the decreased testosterone levels after both drug exposures visualized in this review, mediated by direct effects on Leydig cells or systemic pathways, could lead to damage in BTB.

Impacts on the BTB can also be triggered by free radicals' formation. Our findings confirm the presence of ROS, since the levels of MDA were high after both drug exposures, and the increased levels of peroxynitrite's precursors after finasteride treatment. The membranes from SC and germs cells are rich in polyenoic fatty acids prone to undergo peroxidative decomposition [74]. Lipid peroxidation caused by ROS

can promote a degeneration process, and may contribute to cell death [75]. The control and regulation of cell death programmed events occur through Bcl2, Bax, and p53 proteins, which govern mitochondrial membrane permeability and release cytochrome c for the cytosol [76]. It either triggers the activation of the caspase cascade in the intrinsic apoptotic pathway or amplifies extrinsic apoptotic signaling [77]. Another link between ROS and apoptotic events' occurrence could be through the crosstalk between mitochondria and the ER. When the calcium is forced out from ER by the oxidative stress, its reuptake could happen by mitochondria triggering cell death pathways. Once this happens in the seminiferous tubules, the BTB can be damaged, nonetheless, if the process described occurs in the intertubular space, it may affect the LCs and lead to a drop in testosterone.

In summary, we verified the medicaments relationship between i) the drop in testosterone (by the impact on LCs or HPG axis) and cell death; and the drop in testosterone and BTB damage; ii) the oxidative stress can also cause the same effects; iii) oxidative stress could be the precursor to the drop in testosterone as it can affect the LCs. These hypothetical pathways that we raised in this study, although they cannot be completely described in all their stages, give the first insights into the cellular mechanisms involved in the action of both drugs on the male reproductive system.

Limitations

Although the systematic review has been listed as a high-level study for evaluated studies in a blind way using specific tools [78], there are some limitations. After reviewing all possible pathways outlined in this review, the minoxidil mechanism of action is not well known. It has been supposed that the process could occur through multiple pathways (vasodilatation, anti-inflammatory agent, inducer of the Wnt/b-catenin signaling pathway) [17]. Therefore, could any of these other pathways affect

fertility? We could not answer this question with our findings, but we look forward to further studies.

The bias analysis of the *in vivo* studies also demonstrated some underreported information. For studies that administered finasteride, the primary information not reported was random sequence generation, blinding of participants, and personal and random outcome assessment. For the studies with minoxidil, however, random sequence generation, allocation concealment, blinding of participants and personal, random outcome assessment, and blinding of outcome assessment were not reported. The significant heterogeneity of the data does not indicate that the researchers did not evaluate these parameters but that they have not included them in the reports, which characterizes a high risk of bias. In this case, the most significant limitation among the studies was the lack of information described by the researchers, which hindered the comparison among the studies. Because of this, our initial idea was to make a meta-analysis evaluation impossible to complete. The lack of information in the methodology makes it difficult to replicate the work and diminishes the reliability of the research. Therefore, considering all limitations, we believe that researchers in the reproductive area can use our systematic review to improve the description of the methodology and results for a better reproduction and application of preclinical experiments results that may support clinical trials. Thus, our findings are essential for understanding the mechanism of action of finasteride and minoxidil, describing important points of bias. We hope to contribute to future studies, avoiding those elements of bias that impair the quality of the evidence.

5. Conclusion

We can conclude that finasteride and minoxidil harm the testis and testis-derivate cell cultures. Furthermore, our outcomes revealed that finasteride can damage the epididymis in murine models. It is apparent that both medications act as hormone disruptors, cause oxidative stress, and directly or indirectly affect the BTB in the testicular tissue, thus preventing it from functioning normally. *In vitro*, the comparison was limited to hormonal pathways. There is six times more male repro-toxicology research with finasteride than minoxidil *in vivo* and two times more *in vitro*. Therefore, further studies are needed to assess the effect of minoxidil on the male reproductive system, especially the testis and epididymis.

6. Declarations

6.1 Funding

The Brazilian agencies supported this work: Fundação do Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, processes PPM-00687-17, APQ-03519-22 and APQ-04164-22) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, processes 311105/2020-3,). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – specifically, the Ph.D. scholarships provided for FFVS and AAL.

6.2 Conflict of Interest

The authors declare that they have no conflicts of interest

6.3 Author's contribution statement

FFVS and AAL: data curation; FFVS and JS: Conceptualization, investigation; FFVS, JS, RVG, JS and SLPM: Conceptualization, supervision.

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Declaration of interests

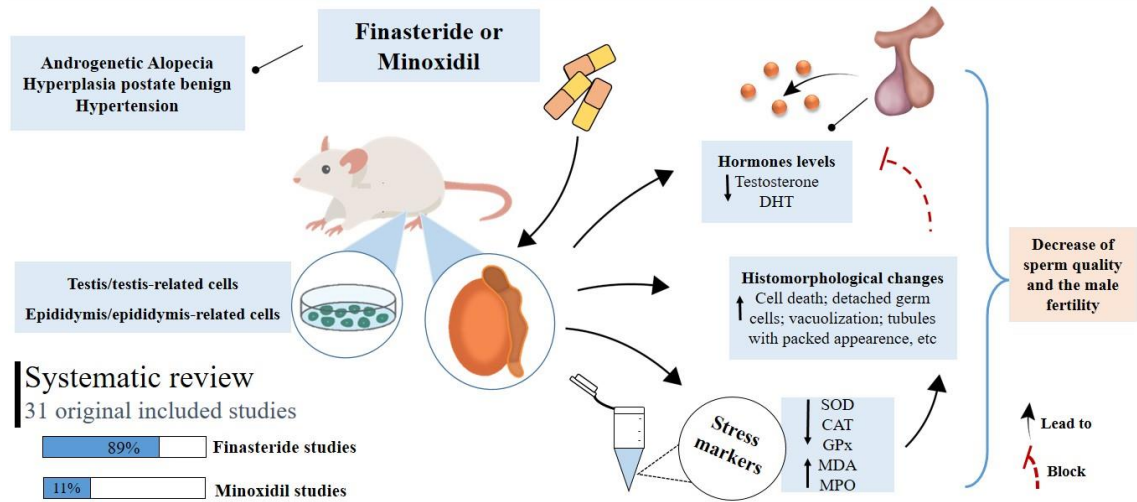
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Francielle Santana reports financial support was provided by Coordination of Higher Education Personnel Improvement.

Graphical Abstract

Comparative effects of finasteride and minoxidil on the male reproductive organs of murine models: A systematic review of *in vivo* and *in vitro* evidence



Highlights

- Finasteride and Minoxidil are approved drugs for androgenetic alopecia
- In the testis the drugs affect lead to male reproductive disorders
- *In vivo* and *in vitro* studies help to make decisions for new therapeutic approaches
- Systematic reviews has been listed as a high-level study