The effects of cannabidiol on male reproductive system: A literature review

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Abstract
Cannabidiol (CBD) is one of the most abundant phytocannabinoids present in the plant Cannabis sativa (marijuana). There have been several studies of CBD in the last few decades, mainly focused on its neuroprotective properties, particularly after the identification of the endocannabinoid system and its participation in the central nervous system. On the other hand, the peripheral effects of CBD, particularly on reproductive physiology, were also evidenced. A narrative review was conducted using the PubMed database to identify studies that analyzed the pharmacological effects of CBD on the male reproductive system of vertebrates and invertebrates. Thirty-two citations (in vivo and in vitro) were identified. Among the vertebrates, the studies were carried out with men, monkeys, rats and mice. Studies with invertebrates are centered exclusively on the sea urchin. The CBD treatment periods include mostly acute and subacute evaluations. Exposure to CBD is associated with a reduction in mammalian testis size, the number of germ and Sertoli cells in spermatogenesis, fertilization rates, and plasma concentrations of hypothalamic, pituitary and gonadal hormones. Moreover, chronic doses of CBD have impaired sexual behavior in mice. From the studies identified in this review, it is possible to conclude that CBD has negative effects on the reproductive system of males. However, knowledge is still limited, and additional research is required to elucidate fully the mechanisms of action, as well as the reversibility of CBD effects on the reproductive system.

KEYWORDS
Cannabis sativa, endocannabinoids, sexual behavior, spermatogenesis, testosterone

1 | INTRODUCTION

Cannabidiol (CBD) is one of several cannabinoids found in marijuana (Cannabis sativa), a plant that contains more than 400 components, about 100 of which are cannabinoids (substances that interact with the endocannabinoid system). CBD constitutes a large part of the chemical composition of the plant and is found in more than 40% of its extracts (Crippa et al., 2009; Taura, Sirikantaramas, Shoyama, Shoyama, & Morimoto, 2007). It is recognized that most actions of cannabimimetic compounds in reproductive physiology may be the result of interactions with cannabinoid receptors (CB1 and CB2) or by inhibition of the enzymatic metabolism of endogenous ligands such as anandamide (Mechoulam & Hanus, 2002). Cannabinoid receptors are coupled to G protein that inhibit adenylate cyclase and therefore reduce Ca2+ currents and stimulate K+ currents (Pertwee, 2002; Rea, Roche, & Finn, 2007) (Figure 1). In the reproductive system, these receptors are present in brain neurons involved in the release of pituitary hormones but are also found in peripheral tissues such as the testes (Pertwee, 1997; Pertwee, 2002; Rossi et al., 2007; Singla, Sachdeva, & Mehta, 2012).

In recent years, the importance of the pharmacological effects of CBD has been recognized, mainly stimulated by the discovery of its...
antiepileptic, antioxidative, anti-inflammatory and neuroprotective actions. This recognition has led to increasing numbers of studies and a growing body of evidence on the action of CBD (Carlini & Cunha, 1981; Carrier, Auchampach, & Hillard, 2006; Chagas et al., 2014; da Silva et al., 2018; Mechoulam, Peters, Murillo-Rodrigues, & Hanus, 2007; Weiss et al., 2006). Previous studies have linked CBD to endocrine physiology, particularly to reproductive physiology (Carvalho, Santos, et al., 2018; Daltorio, Bartke, & Mayfield, 1983; Jakubovic, McGee, & McGee, 1979; Murphy, Steger, Smith, & Bartke, 1990; Steger, Murphy, Bartke, & Smith, 1990). A comprehensive review of these studies, particularly from the point of view of male reproductive biology, has not been undertaken so far. Thus, this review aims to meet this demand.

The present review encompasses the CBD interaction with reproduction and development. Thus, the interaction can occur: (1) at a genetic level, programmed during differentiation and expressed at puberty; (2) through inducing enzymes that metabolize endocannabinoids; or (3) as competing substrates for a given enzyme (Daltorio & deRooij, 1986; Harclerode, Nyquist, Nazar, & Lowe, 1979; Leweke et al., 2012; List, Nazar, Nyquist, & Harclerode, 1977).

1.1 Cannabis plant

Plants of the genus Cannabis are generally classified as Cannabis sativa, regardless of their origin. However, according to McPartland, Clarke and Watson (2000), it can present some varieties and be categorized as Cannabis sativa of varieties: sativa, indica, afghanica and ruderalis, among others. Each of the varieties has different characteristics such as color, size, leaf quantity, inflorescences and delta-9-tetrahydrocannabinol (Δ9-THC) and another cannabinoid content. It is an annual dioecious plant that is perpetuated by means of seeds.
and can grow in fertile soils and in degraded areas of tropical and temperate regions but does not tolerate frost (Markez, 2002; Stambouli, El Bouri, Bellimam, Bouayoun, & El Karmi, 2005).

Cannabis is among the oldest herbs grown by humans. The first evidence of its use was found in Asia about 6000 years ago, being cultivated by the Chinese in the production of fibers to produce ropes, fabrics and paper. Seed was used as food and its use as a drug was reported in the Chinese pharmacopoeia (Li, 1974).

At the end of the nineteenth century, therapeutic use became very popular in the West. At that time, the main psychoactive component of marijuana, Δ⁹-THC, was being analyzed (Fankhauser, 2002; Hampson & Deadwyler, 1999; Solowij & Grenyer, 2002). In the twentieth century, the recreational use of cannabis spread rapidly among the younger population in the Western world, particularly in the USA. Owing to the psychoactive effects leading to cognitive alteration and episodes of dependence, marijuana came to be viewed as a social problem. Thus, its use was restricted by the mid-1930s in the USA and other countries, limiting medical use and experimentation with Cannabis (Grinspoon & Bakalar, 1993). In 1964, the chemical structure of Δ⁹-THC was identified by Raphael Mechoulam and Yechiel Gaoni, which contributed to a proliferation of research into Cannabis and its efficacy and safety as a therapeutic drug (Carlini, Santos, Claussen, Bieniek, & Korte, 1970; Gaoni & Mechoulam, 1964; Lehmann et al., 2016; Welty, Luebke, & Gidal, 2014; Zuardi, Cosme, Graeff, & Guimarães, 1993).

### 1.2 | Phytocannabinoids

Phytocannabinoids, also known as exogenous cannabinoids, are active and chemically related compounds present in the resin glands located at the tip of the secretory hairs, mainly in the female Cannabis plant (Touwn, 1981). These compounds are formed by the decarboxylation of 2-carboxylic acids, a process that is catalyzed by conditions of heat, light or alkalinity. They are also highly lipophilic and essentially insoluble in water (Garrett & Hunt, 1974).

The mechanisms of action of exogenous cannabinoids are practically the same for men and women, as well as for frequent and infrequent users. Pharmacokinetics is best elucidated based on studies with Δ⁹-THC (Kelly & Jones, 1992; Wall, Sadler, Brine, Taylor, & Perez-Reyes, 1983). Generally, they are ingested by means of capsules, in liquids and foods or smoked. Other methods include aerosols or inhalation with vaporizers (to avoid the damage associated with smoking) and as eye drops to decrease intraocular pressure. Various other forms and routes of administration have been tested for therapeutic purposes. The rectal route with suppositories has been applied in some patients and the dermal, sublingual, parenteral, intravenous and intramuscular routes are employed in laboratory studies or in isolated examples of therapeutic use (Brenneisen, Egli, Elsohen, Hen, & Spiess, 1996; Dogrul et al., 2003; Lichtman et al., 2000; McGuigan, 2006; Tomida et al., 2006; Uribe-Mariño et al., 2012).

With inhalation by smoking or vaporization, some phytocannabinoids are detected in the plasma within seconds, with maximum plasma concentrations in 3-10 minutes (Huestis, Henningfield, & Cone, 1992). Oral absorption is slow and erratic, resulting in maximum plasma concentrations after 60-120 minutes, but they have also been observed 4 and 6 hours after oral use (Agurell, Lindgren, Ohlsson, Gillespie, & Hollister, 1984).

Although a large part of the absorption of Δ⁹-THC phytocannabinoid occurs in the upper region of the small intestine (90%-95%), on average only 6% will be bioavailable due to extensive hepatic biotransformation resulting from the first-pass effect in the liver. In addition, part of the dose of Δ⁹-THC undergoes degradation both in the stomach, due to its acidity and in the intestine, by the action of the microbiota (Moreau, 1996). In a study with rabbits, systemic bioavailability varied between 6% and 40% with ophthalmic exposure. Plasma concentrations peaked after 1 hour and remained high for several hours (Chiang, Barnett, & Brine, 1983). Tissue distribution occurs through physicochemical properties, without barriers or specific transport processes that affect the tissue concentration of phytocannabinoids (Leuschner, Harvey, Bullingham, & Paton, 1986). About 90% of exogenous cannabinoids are distributed in plasma and 10% in red cells. In plasma, the bound fraction of cannabinoids is 95%-99%, being mainly bound to lipoproteins (Fehr & Kalant, 1974).

Metabolism occurs primarily in the liver through microsomal hydroxylation and enzyme catalyzed oxidation of the cytochrome P-450 complex, but other tissues are also able to metabolize phytocannabinoids, including the heart and lungs, but to a lesser degree (Harvey & Paton, 1976).

Elimination occurs mainly as acid metabolites and was detected for 3.5 days after a low dose and 6.3 days after smoking at a high dose (Huestis et al., 1992). The main reason for the long plasma elimination period is the prolonged redistribution of body fat and other tissues to the blood (Leuschner et al., 1986). The elimination half-life of the metabolites is longer than the elimination half-life of the parent molecule in plasma. About 65%-80% are eliminated in feces (with less than 5% as unchanged drug after an oral dose) and 20%-35% in urine. A global excretion rate of about 65% after oral administration and about 45% following intravenous administration can be observed after 3 days (Wall et al., 1983).

### 1.3 | Endogenous cannabinoid system

The endogenous cannabinoid system, also known as the endocannabinoid system, is a biochemical endogenous signaling system that comprises the cannabinoid receptors, the endogenous cannabinoids (endocannabinoids, such as anandamide), enzymes involved in endocannabinoid metabolism and the membrane endocannabinoid transporter (Fonseca, Costa, Almada, Correia-da-Silva, & Teixeira, 2013).

Only two types of cannabinoid receptors have been described, the CB1 receptor that was cloned in 1990 and the CB2 receptor, cloned in 1993 (Pertwee, 1997). Cannabinoid receptors are coupled to an inhibitory G protein (Figure 1), which, when activated, inhibits the adenylate cyclase enzyme, leading to a decrease of the intracellular
concentration of cyclic adenosine monophosphate with inhibition of calcium entry and cellular hyperpolarization by opening potassium channels (Pertwee, 2002; Rea et al., 2007) (Figure 1). Activation of cannabinoid receptors by agonists also promotes alteration of gene expression, either directly by the activation of mitogen-activated protein kinase or indirectly by the reduction of protein kinase A activity, because of the reduced activity of adenylate cyclase (Rang, Flower, Henderson, & Ritter, 2016).

CB1 receptors are mainly located in central presynaptic neurons but are also found in some peripheral organs and tissues such as the heart, arteries, spleen, white blood cells, endocrine glands and parts of the reproductive, gastrointestinal and urinary tract. CB2 receptors are found mainly in white blood cells and peripheral tissues such as the spleen and tonsils, but they can also be expressed in cells of the reproductive system, such as the Sertoli cells (Pertwee, 1997; Pertwee, 2002; Rossi et al., 2007; Singla et al., 2012).

Endogenous cannabinoids are lipophilic signaling molecules widely distributed in the body (Garrett & Hunt, 1974). These molecules meet the criteria as neurotransmitters, but, unlike classical neurotransmitters, they are synthesized in post-synaptic neurons according to demand and are not stored in vesicles (Malone, Jongejan, & Taylor, 2009). This mechanism produces a backward traffic of the neuronal information from the post-terminals to the presynaptic, where they bind to the cannabinoid receptors (Brown, Brotchie, & Fitzjohn, 2003; Piomelli, 2003).

To date, five endocannabinoids have been identified: 2-arachidonoyl glycerol (2-AG); N-arachidonoyl ethanolamide (anandamide); 2-arachidonoyl glyceryl ether (noladin ether); O-arachidonoyl ethanol amine (virodhamine); and N-arachidonoyl-dopamine. Anandamide was detected in 1992 and, along with 2-AG, are the most exploited endocannabinoids (Hanuš et al., 2001; Mechoulam et al., 1995; Porter et al., 2002). The bioavailability of endocannabinoids is regulated by the uptake-degradation of different enzymes. Anandamide, 2-AG and several other endogenous cannabinoids are derived from arachidonic acid, which is one of the unsaturated fatty acids found in cell membrane phospholipids (Malone et al., 2009).

Anandamide is part of the N-acyl ethanolamines group and can be stored in N-arachidonoyl phosphatidylethanolamine (NAPE) membranes through the enzyme N-acyl transferase. The enzyme N-acyl-phosphatidyl-ethanolamine-phospholipase D is then responsible for converting NAPE to anandamide. 2-AG formation is also initiated by neuronal activity accompanied by an increase in intracellular calcium concentrations. Biosynthesis occurs through the hydrolysis of membrane phospholipids by phospholipase C, which produces diacylglycerol, which is then converted to 2-AG by the enzyme 1,2-diacylglycerol lipase (Freund, Katona, & Piomelli, 2003).

Like other neurotransmitters, endocannabinoids are rapidly inactivated after production and release, and transported to the intracellular medium by the membrane endocannabinoid transporter or by simple diffusion. 2-arachidonoyl glycerol is transported to presynaptic neurons where it is hydrolyzed, mainly by the monoacylglycerol lipase enzyme, which converts 2-AG to arachidonic acid and glycerol (Dinh, Freund, & Piomelli, 2002; Hermann, Kaczocha, & Deutsch, 2006). The anandamide is transported back to the postsynaptic neurons and then hydrolyzed to arachidonic acid and ethanolamine by means of the fatty acid amide hydrolase enzyme (FAAH) (Fowler, 2006) (Figure 1).

Anandamide and the set of endocannabinoids play important roles in many biological processes. These substances act physiologically in the functioning of the organism at the central and peripheral levels, influencing nerve transmission, interacting with multiple systems and neurotransmitters, and preserving body homeostasis (Di Marzo, Bifulco, & De Petrocellis, 2004; Kendall & Yudowski, 2017; Marsicano & Lutz, 2006). In the vertebrate reproductive system, anandamide has been reported to modulate sexual behavior, presenting a biphasic response: small amounts stimulate sexual activity, while high doses inhibit it (Canseco-Alba & Rodríguez-Manzo, 2014).

1.4 | Cannabidiol

CBD is one of the most abundant phytocannabinoids present in the C. sativa plant. CBD was isolated in the early 1940s (Adams, Hunt, & Clark, 2010); however, its chemical structure was only identified in 1963 by Raphael Mechoulam et al. Over the years, Mechoulam's research group was responsible for determining the structure and stereochemical characteristics of the main cannabinoids present in cannabis, including CBD, which encouraged the investigation of the pharmacological functions of these compounds (Mechoulam, 1973; Mechoulam & Shvo, 1963).

The observation that CBD could antagonize some of the major pharmacological effects of Δ²-THC led to the hypothesis that CBD could present an anxiolytic action as well as an antipsychotic profile (Zuardi, Shirakawa, Finkelfarb, & Karniol, 1982). The first pharmacological actions described were as antiepileptics and sedatives. In 1973, a Brazilian group led by Elsaldo Carlini reported that CBD was active in reducing or blocking induced seizures in laboratory animals in several models (Carlini, Leite, Tannahouser, & Berardi, 1973; Izquierdo, Orsingher, & Berardi, 1973). This hypothesis continues to be tested in different animal models (Almeida et al., 2013; Campos & Guimarães, 2008). Carlini and his team of researchers have been contributing to medical marijuana studies, enabling the development of drugs from the C. sativa plant, such as CBD, used in several countries to treat epilepsy and multiple sclerosis.

It is now known that CBD has multiple mechanisms of action and that the various pharmacological effects arise from its inhibitory action on the endocannabinoid uptake and degradation system (particularly on the hydrolytic enzyme FAAH), accumulating substrates of the endocannabinoid system, such as anandamide (Figure 1), in the ligand sites (Leweke et al., 2012).

CBD is a CB1 and CB2 antagonist and there is evidence showing its agonist action on the transient receptor potential cation channel subfamily V member 1 (TRPV1) with the same efficacy as that obtained by capsaicin, a natural agonist of this receptor. TRPV1 is a non-selective cation channel, and its activation triggers a membrane depolarization wave (Figure 1) and, consequently, the opening of
voltage-operated calcium channels (Bernabò et al., 2012; Bisogno et al., 2001).

2 | MATERIALS AND METHODS

This study is a narrative review of articles (in vivo and in vitro) that evaluated the effects of CBD on male reproductive function of vertebrates and invertebrates. A PubMed search was performed for articles from 1977 to 2018 and for articles with English language. In this review, citations (32) were selected using the following terms: “male reproductive system and cannabidiol”; “testes and cannabidiol”; “epididymis and cannabidiol”; “vas deferens and cannabidiol”; “prostate and cannabidiol”; “semenal vesicle and cannabidiol”; “testosterone and cannabidiol”; “luteinizing hormone and cannabidiol”; “Sertoli cells and cannabidiol”; “Leydig cells and cannabidiol”; “sperm and cannabidiol”; “fertility and cannabidiol”; and “sexual behavior and cannabidiol.” Emphasis was placed on contents aimed at the male sexual effects.

3 | RESULTS AND DISCUSSION

Over the last few decades, several studies have reported that CBD has neuroprotective properties. Pharmacological uses in the treatment of anxiety, depression, Alzheimer’s disease and Parkinson’s disease have been reported. In addition, it has also been shown that CBD is useful in reducing the severity of seizures and is therefore effective in the treatment of children and adolescents with drug-resistant epilepsy.

Because of the increased use of CBD, we reviewed the effects of this phytocannabinoid on the male reproductive system to show the potential impacts of acute and chronic exposure to CBD, and to contribute to reproductive safety in front of the consumption of this medicine.

### TABLE 1  Cannabidiol effects on cellular and enzymatic metabolism in testes and hepatic microsomes

<table>
<thead>
<tr>
<th>Mammals</th>
<th>Exposure conditions</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Concentration (mg/kg)</td>
<td>Time</td>
<td>Route/method</td>
</tr>
<tr>
<td>Rat</td>
<td>0.1 mM</td>
<td>90 min</td>
<td>Incubation</td>
</tr>
<tr>
<td>Rat</td>
<td>2</td>
<td>10 days</td>
<td>i.p.</td>
</tr>
<tr>
<td>Rat</td>
<td>2, 10</td>
<td>6 h, 10 days</td>
<td>i.p.</td>
</tr>
<tr>
<td>Rat</td>
<td>30 μM 10</td>
<td>5 min 3 days</td>
<td>Incubation</td>
</tr>
<tr>
<td>Mouse</td>
<td>130 μM 120</td>
<td>10 min 2 h, 4 days</td>
<td>Incubation</td>
</tr>
<tr>
<td>Rat</td>
<td>10</td>
<td>1 day</td>
<td>i.p.</td>
</tr>
<tr>
<td>Rat</td>
<td>10, 100, 1000 μM</td>
<td>20 min</td>
<td>Incubation</td>
</tr>
<tr>
<td>Rat</td>
<td>1, 10, 50, 100, 200, 300 μM</td>
<td>2 min</td>
<td>Incubation</td>
</tr>
</tbody>
</table>

ADP, adenosine diphosphate; ATP, adenosine triphosphate; OHT, hydroxytestosterone; i.p., intraperitoneal; p.o., per os (oral).
Tables 1–5 show data about the effects of CBD on the male reproductive system, such as effects on: cellular and enzymatic metabolism (Table 1); the reproductive endocrine system (Table 2); reproductive organs (Table 3); spermatogenesis (Table 4); and sperm morphology, sexual behavior and fertility (Table 5). Among the vertebrates (88%), the studies were carried out with mice (38%), rats (44%), monkeys (3%) and men (3%). Studies with invertebrates (12%) are centered exclusively on the sea urchin. It is interesting to note that the number of in vivo and in vitro studies was similar, and the CBD treatment periods include mostly acute (61%) and subacute (26%) evaluations.

### 3.1 Effect on cellular and enzymatic metabolism: hepatic and testicular

Hepatic metabolism of steroid hormones, such as testosterone, is performed by thecytochrome P-450-dependent oxidative system (Ryan & Levin, 1990). This enzymatic system is a major way by which a living organism can convert lipophilic substances, including androgens, into more water-soluble products, thereby facilitating their elimination from the body. These alterations are usually grouped into two types of metabolism, phase I and phase II. Phase I reactions generally convert the steroid by enzymatically catalyzed reactions.

#### TABLE 2 Cannabidiol effects on reproductive endocrine system

<table>
<thead>
<tr>
<th>Mammals</th>
<th>Concentration (mg/kg)</th>
<th>Time</th>
<th>Route/method</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>2, 10</td>
<td>6 h</td>
<td>i.p.</td>
<td>T was decreased by a single 10 mg/kg dose.</td>
<td>List et al., 1977</td>
</tr>
<tr>
<td>Rat</td>
<td>2, 10</td>
<td>24 h</td>
<td>i.p.</td>
<td>T was decreased by a single 10 mg/kg dose.</td>
<td>Harclerode et al., 1979</td>
</tr>
<tr>
<td>Rat</td>
<td>0.15, 1.5, 15, 150 μM</td>
<td>3 h</td>
<td>Incubation</td>
<td>T was decreased after 1.5-150 μM doses.</td>
<td>Jakubovic et al., 1979</td>
</tr>
<tr>
<td>Rat</td>
<td>? a</td>
<td>18 h</td>
<td>Incubation</td>
<td>Reduced binding DHT to the androgen receptor.</td>
<td>Purohit et al., 1980</td>
</tr>
<tr>
<td>Monkey</td>
<td>30, 100, 300</td>
<td>90 d</td>
<td>p.o.</td>
<td>T was decreased after treatment by higher dose. LH was elevated at higher doses after recovery (30 days after cessation of treatment). FSH increased after treatment and remained elevated at the higher doses after recovery.</td>
<td>Rosenkrantz &amp; Esber, 1980</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>5 w</td>
<td>p.o.</td>
<td>No effect on T, LH and FSH.</td>
<td>Dalterio et al., 1982</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.25, 2.5, 25, 250, 2500, 25 000 ng/mL, 0.25, 2.5 μg</td>
<td>4 h, 1 h</td>
<td>Incubation, Intratesticular injection</td>
<td>T: in vitro was increased at 2.5 and reduced at 2500-25 000 ng/mL; in vivo was increased at 2.5 μg.</td>
<td>Dalterio et al., 1983</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>1 d</td>
<td>p.o.</td>
<td>No marked change in T (plasmatic or testicular) and FSH concentrations of intact and castrated mice. LH: no effect in intact mice but increased after T injection in castrated mice.</td>
<td>Dalterio et al., 1984a</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>1 d</td>
<td>p.o.</td>
<td>Testicular T: in intact mice was reduced; in response to hCG stimulation in vitro was increased and after intratesticular hCG administration was reduced. LH: was reduced in intact and post-castration mice. FSH: no effect.</td>
<td>Dalterio et al., 1984b</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>1 d</td>
<td>p.o.</td>
<td>T and FSH: No effect. LH: reduced in mice exposed on day 1 postpartum.</td>
<td>Dalterio et al., 1986</td>
</tr>
<tr>
<td>Rat</td>
<td>0.5</td>
<td>30, 60, 120 min</td>
<td>p.o.</td>
<td>LH: only CBD, no effect; CBD + Δ⁹-THC, decreased at each time interval. PRL: no effect.</td>
<td>Murphy et al., 1990</td>
</tr>
<tr>
<td>Rat</td>
<td>0.1, 1, 10</td>
<td>1 h</td>
<td>p.o.</td>
<td>T, LH, FSH, LHRH: no effect.</td>
<td>Steger et al., 1990</td>
</tr>
<tr>
<td>Mouse</td>
<td>15, 30</td>
<td>34 d</td>
<td>p.o.</td>
<td>T was reduced by higher dose.</td>
<td>Carvalho, Santos, et al., 2018</td>
</tr>
<tr>
<td>Mouse</td>
<td>15, 30</td>
<td>34 d</td>
<td>p.o.</td>
<td>P: no effect.</td>
<td>Carvalho, Souza, et al., 2018</td>
</tr>
</tbody>
</table>

CBD, cannabidiol; DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LHRH, LH releasing hormone; P, progesterone; PRL, prolactin; T, testosterone; Δ⁹-THC, delta-9-tetrahydrocannabinol; i.p., intraperitoneal; p.o., per os (oral).

Dose not specified.
### TABLE 3  Cannabidiol effects on sexual organs weight

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration (mg/kg)</th>
<th>Time</th>
<th>Route/method</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>2</td>
<td>10 days</td>
<td>i.p.</td>
<td>Testes weight was reduced.</td>
<td>Goldstein et al., 1977</td>
</tr>
<tr>
<td>Rat</td>
<td>0.6, 0.8, 1.2</td>
<td>17 days</td>
<td>Inhalation</td>
<td>Testes weight was reduced by high dose.</td>
<td>Rosenkrantz &amp; Hayden, 1979</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>5 weeks</td>
<td>p.o.</td>
<td>No effect in testes weight.</td>
<td>Dalterio et al., 1982</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>1 day</td>
<td>p.o.</td>
<td>Testes and seminal vesicle weight were increased in adult offspring.</td>
<td>Dalterio et al., 1984a</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>1 day</td>
<td>p.o.</td>
<td>Testes weight of adult offspring was reduced.</td>
<td>Dalterio et al., 1984b</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>15, 35 days</td>
<td>p.o.</td>
<td>No effects in testes, ventral prostate, epididymis and seminal vesicle.</td>
<td>Patra &amp; Wadsworth, 1991</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.316, 1, 3.16, 6, 10 μM</td>
<td>30 min</td>
<td>Incubation</td>
<td>Presented antagonistic action on cannabinoid receptor agonists (all doses) and norepinephrine (at 10 μM); increased the amplitude of ATP-mediated contractions (at 3.16-10 μM) in the vas deferens.</td>
<td>Pertwee et al., 2002</td>
</tr>
<tr>
<td>Mouse</td>
<td>15, 30</td>
<td>34 days</td>
<td>p.o.</td>
<td>No effects in testes, epididymis and seminal vesicle.</td>
<td>Carvalho, Santos, et al., 2018</td>
</tr>
</tbody>
</table>

ATP, adenosine triphosphate; i.p., intraperitoneal; p.o., per os (oral).

### TABLE 4  Cannabidiol effects on testicles and spermatogenesis

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration (mg/kg)</th>
<th>Time</th>
<th>Route/method</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.6, 0.8, 1.2</td>
<td>17 days</td>
<td>Inhalation</td>
<td>Promoted degeneration of ST and inhibition of sperm maturation.</td>
<td>Rosenkrantz &amp; Hayden, 1979</td>
</tr>
<tr>
<td>Human</td>
<td>636 nM</td>
<td>7 days</td>
<td>Incubation</td>
<td>No effect in the incubated S.</td>
<td>Holmes et al., 1983</td>
</tr>
<tr>
<td>Mouse</td>
<td>1, 3, 5 μM</td>
<td>2 h</td>
<td>Incubation</td>
<td>Suppressed the incorporation of [3H]-uridine by incubated P and RS at doses 3-5 μM.</td>
<td>Tilak &amp; Zimmerman, 1984</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>1 day</td>
<td>p.o.</td>
<td>ES was reduced on day 1 postpartum.</td>
<td>Dalterio &amp; deRooij, 1986</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>15, 35 days</td>
<td>p.o.</td>
<td>L: LCNA was greater after 15 treatments. ST: tubular cells reduced to a single layer; vacuole formation at the periphery of the tubules; enlarged lumen; desquamation and accumulation of germ cells within the lumen of the tubules; several tubules were shrunken and atrophied. A: reduced in stages I, II, VII, IX and XI; P: pyknosis among cells; quantity was reduced after 15 days treatment, as well as resting spermatocytes amount. However, after 35 days these cell types were normal. RS: multinuclear giant cell formation within the nest of the spermatids.</td>
<td>Patra &amp; Wadsworth, 1991</td>
</tr>
<tr>
<td>Rat</td>
<td>0.8, 1.6, 3.1 μg/mL</td>
<td>48 h</td>
<td>Incubation</td>
<td>The highest dose stimulated lactate production by S cultured in serum-containing media.</td>
<td>Newton et al., 1993</td>
</tr>
<tr>
<td>Mouse</td>
<td>15, 30</td>
<td>34 days</td>
<td>p.o.</td>
<td>S: reduced in stages XII by higher dose. ST: increased stages I-VI and reduced stages VII-VIII and XII; seminiferous epithelium presented an increase in height; lower dose increased perimeter of the tubules and area of the epithelium.</td>
<td>Carvalho, Santos, et al., 2018</td>
</tr>
</tbody>
</table>

A, type A spermatagonia; ES, elongated spermatid; L, Leydig cell; LCNA, Leydig cell nuclear area; P, pachytene spermatocytes; RS, round spermatid; S, Sertoli cell; ST, seminiferous tubule; p.o., per os (oral).
Phase II reactions, which are also called conjugation reactions, act to couple the steroid or its metabolite with glucuronic acid or sulfate, aiding in hormone elimination (Schänzer, 1996). In vivo and in vitro studies also showed interactions of CBD in the activity of cytochrome P-450 enzymes, involved in the hepatic degradation of gonadal steroids (Table 1).

In liver microsomes of male mice, Bornheim and Correia (1989) found that in vitro treatment with CBD resulted in the reduction of cytochrome P-450 content. In vivo treatment with a single dose, with the animals being killed 2 hours later, also reduced cytochrome P-450 content. The authors also reported a reduction in the metabolites 16α- and 6β-hydroxytestosterone (16α- and 6β-OHT), produced by the catabolism of testosterone, which corresponds to the reduction in cytochrome P-450 isoenzymes. On other hand, repeated doses of CBD, with or without an additional dose (2 hours before killing), resulted in no change in cytochrome P-450 content. A consistent explanation for this paradox is that CBD inhibits a constitutive cytochrome P-450 isozyme(s) responsible for the metabolism in both (in vitro and acute in vivo) treatments, whereas repetitive CBD administration results in the increase of a different cytochrome P-450 isozyme (Figure 2). Narimatsu, Watanabe, Yamamoto, and Yoshimura (1988) evaluated the in vivo and in vitro effects of CBD exposure on male rat hepatic microsomes. The data showed selective action of CBD on cytochrome P-450 enzymes. The formation of the testosterone metabolites 2α- and 16α-OHT was reduced in both treatments. Androstenedione formation activity was significantly suppressed and the 7α-OHT metabolite was increased in vivo (Figure 2).

In another in vivo study, rats were treated with a single dose of CBD and killed 6, 12, 24, 48, 72 hours and 1 week after treatment. The time course results revealed that the 2α- and 16α-OHT metabolites, as well as androstenedione, were suppressed within 6–48 hours after CBD exposure. Formation of the 6β-OHT metabolite was suppressed only 6 hours after CBD administration, and returned to control levels after 24 hours (Narimatsu et al., 1990). According to the authors, all these reactions were catalyzed by a male-specific cytochrome P-450 (P4502C11 or P450h), which was markedly decreased following CBD administration, reaching the maximum values after 24–48 hours. A similar response was shown using an in vitro protocol with hepatic microsomes from male rats exposed to CBD (Watanabe et al., 2005). On the other hand, CBD administration

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**TABLE 5** Cannabidiol effects on male sexual behavior, sperm morphology, fertilizing capacity and number of pups

<table>
<thead>
<tr>
<th>Species</th>
<th>Exposure conditions</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>10, 25</td>
<td>5 days i.p.</td>
<td>No effect on sperm morphology.</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>5 weeks p.o.</td>
<td>Adult male mice impregnated fewer females and their offspring showed more prenatal and postnatal deaths.</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>1 day p.o.</td>
<td>Male offspring exposed postnatally (12 h after parturition) impregnated fewer females in adulthood; live pups were reduced by adult male offspring exposed prenatally (12th day of gestation) and postnatally.</td>
</tr>
<tr>
<td>Sea urchin</td>
<td>0.1, 0.5, 1, 10 μM</td>
<td>5 min Incubation</td>
<td>Incubated sperm did not fertilize eggs.</td>
</tr>
<tr>
<td>Mouse</td>
<td>50</td>
<td>15, 35 days p.o.</td>
<td>Head abnormalities of sperm were increased at 35 days of treatment. The 15-day treatment produced some abnormal spermatozoa although its effect was not statistically significant.</td>
</tr>
<tr>
<td>Sea urchin</td>
<td>0.1, 0.5, 10 μM</td>
<td>5 min Incubation</td>
<td>Incubated sperm did not fertilize eggs.</td>
</tr>
<tr>
<td>Sea urchin</td>
<td>100 μM</td>
<td>10 min Incubation</td>
<td>Incubated sperm blocked the acrosome reaction and produced electron-dense lipid deposits within the sub-acrosomal and centriolar fossae.</td>
</tr>
<tr>
<td>Sea urchin</td>
<td>100 μM</td>
<td>5 min Incubation</td>
<td>Incubated sperm produced a reduction in the acrosome reaction elicited by stimulation with egg jelly and inhibited the spontaneous acrosome reaction.</td>
</tr>
<tr>
<td>Mouse</td>
<td>15, 30</td>
<td>34 days p.o.</td>
<td>Head abnormalities of sperm were increased.</td>
</tr>
<tr>
<td>Mouse</td>
<td>15, 30</td>
<td>34 days p.o.</td>
<td>Lower dose promoted impairment while the higher dose promoted an improvement in sexual performance; fertility rate and number of pups was reduced by higher dose.</td>
</tr>
</tbody>
</table>

i.p., intraperitoneal; p.o., per os (oral).
in an acute protocol (animals being killed 6 hours later), or short-repeated exposure induced activation of the cytochrome P-450 enzyme, resulting in an elevation in testosterone hydroxylation (List et al., 1977).

The effects of CBD on protein synthesis and esterase isoenzymes in rat testes were also demonstrated (Table 1). Jakubovic and McGeer (1977) treated rat testicular tissues in vitro with CBD and found a decrease in adenine nucleotide synthesis. The data also indicated that the total concentration of adenosine diphosphate and adenosine triphosphate (ATP) in the testicular tissue decreased. According to the authors, these findings suggest a possible mechanism by which CBD can directly interfere in the cellular synthetic process. In this sense, the reduction in ATP concentration may be related to the reduction of nucleic acid precursors, and, consequently, in the decrease of protein synthesis. In another study, CBD-treated rats showed a reduction in one of the esterase isoenzymes present in Leydig cells (Goldstein, Harclerode, & Nyquist, 1977). The authors correlated this effect with a reduction in the secretion of luteinizing hormone (LH) promoted by the treatment, as the development and maintenance of Leydig cells are dependent on LH. However, it is also possible that CBD interfered directly with enzyme activity, just as it did in protein synthesis.

In testicular microsomes of rats, in vivo treatment with a single dose of CBD promoted a reduction in testosterone production (List et al., 1977). In vitro studies have also shown the effects of CBD on the activity of the progesterone 17α-hydroxylase enzyme (one of the steroidogenic enzymes) (Funahashi et al., 2005; Watanabe et al., 2005). The authors observed that doses above 100 μM inhibited enzymatic activity and reduced the conversion of progesterone to 17α-hydroxyprogesterone (Figure 3). These findings highlight the

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**FIGURE 2** Schematic drawing of the action of cannabidiol on cytochrome P-450 isozymes, responsible for the hepatic hydroxylation of testosterone. Formation of the 2α-OHT metabolite is catalyzed by the isozymes P450b and P450h; 6β-OHT is formed from the action of the isozymes P450c, P450d, P450g, P450h, P450k and P450p; 7α-OHT is formed by the action of isozyme P450a; 16α-OHT and AD are formed by the isozymes P450b, P450e and P450h. Increased formation pathway of the 7α-OHT (solid arrow) metabolite may be a result of the reduction in the other routes of testosterone hepatic metabolism (dotted arrows). OHT, hydroxytestosterone; AD, androstenedione.

**FIGURE 3** Action of CBD on 17α-hydroxylase enzyme in rat testes. CBD inhibited the activity of 17α-hydroxylase enzyme (dotted arrow), responsible for the conversion of progesterone into 17α-hydroxyprogesterone. This effect of CBD might result in the reduction of testicular testosterone synthesis in rats. Testosterone is synthesized from cholesterol by a sequence of enzymatic chains, mainly in the Leydig cells, located in the interstitium of the mature testes. CBD, cannabidiol; 17α-OH-lase, 17α-hydroxylase; 17α-OH-progesterone; 17α-hydroxyprogesterone.
importance of evaluating androgen levels and hypothalamic-pituitary-gonad (HPG) axis integrity evaluation during chronic treatment with CBD.

In general, CBD exerts an inhibitory effect not only on hepatic degradation of sexual steroids, but also on testicular synthesis. Although the precise mechanisms of how CBD acts on androgen metabolic modulation are still unknown, the endocannabinoid system is known to be present in the testes and liver (Alsawat, 2013; Rossi et al., 2007). Thus, CBD can act directly on the tissues through an interaction with the endogenous cannabinoid system promoting the described effects. In this way, the results of CBD studies in the liver of rats and mice point to a modulation in androgen metabolism.

Another important point to note is the specific determination of cytochrome P-450 isozymes. In understanding that several CBD users also consume other medications, it is important that new studies identify which isoenzymes of the P-450 system are metabolic pathways for this phytocannabinoid, to avoid a drug interaction potentiating the negative effect of CBD on steroidogenesis.

### 3.2 Effects on reproductive endocrine system

Added to changes in androgen metabolism, alterations in sex hormone concentrations following exposure to CBD were reported by several authors (Table 2). In vitro treatment with Leydig cells from rats showed a reduction in testosterone concentrations (Jakubovic et al., 1979). In another in vitro study, incubation of the ventral prostate of rats with CBD (unspecified dose) showed reduced binding of the hormone dihydrotosterone to its receptor (Purohit, Ahlawat, & Vigersky, 1980). In vivo studies with rats have shown divergent results. In acute and repeated administration of CBD, List et al. (1977) found testosterone reduction during acute injection. The same result was found in the study by Harclerode et al. (1979), who administered a single dose of CBD to a group of rats, killed 24 hours later; as well as repeated doses to another group of animals for 10 days.

Although these studies in rats showed a change in testosterone concentration, only with single intraperitoneal administration the dose used was five times higher when compared with repeated administrations, which may have contributed to the observed changes. On the other hand, oral doses of a single administration of CBD did not promote changes in the hormones, testosterone, LH, follicle-stimulating hormone (FSH) and luteinizing hormone releasing hormone in rats (Steger et al., 1990). However, the bioavailability of CBD may have been reduced by the first passage through the liver, due to the route of administration used (oral), which ensured normality in the hormonal concentrations. In fact, in rats killed 30, 60 or 120 minutes after administration of a single oral dose of CBD, there was no change in LH and prolactin. However, after the addition of Δ⁹-THC at the same dosage, the LH concentration was reduced at all the time intervals analyzed (Murphy et al., 1990). Δ⁹-THC is known to reduce LH plasma levels in rats (Wenger, Rettori, Snyder, Dalterio, & McCann, 1987) and its addition potentiated cannabinoid action on gonadotropin concentrations.

Hormonal assessments after exposure to CBD were also performed in mice. Dalterio, Badr, Bartke, and Mayfield (1982) did not find significant changes in the amount of testosterone, LH and FSH in mice treated for 5 weeks (3 days/week) with oral doses of CBD. The hormonal concentrations of testosterone (Carvalho, Santos, et al., 2018) and progesterone (Carvalho, Souza, et al., 2018) in mice treated with oral doses for 34 days, after a period of 35 days of recovery was recently evaluated. The results showed that progesterone and testosterone concentrations were within the normal reference range, even with a significant 76% reduction in testosterone concentrations in animals treated with CBD. The divergence with the result of the previous study on testosterone plasma concentrations may be explained by the greater frequency (34 consecutive days) of administration, even if a lower dosage was used. In addition, an age-dependent reaction may have occurred. While the first study exposed adult mice to CBD, the latter started exposure at 21 days post-birth when the animals were not sexually mature. Exposure during this period may have contributed to the reduction in androgen. On the other hand, intratesticular administration (in vivo) and incubation of testicular tissue (in vitro) with CBD, in mice, showed that a single intratesticular administration, as well as the in vitro administration, promoted an increase in testosterone concentrations. However, high doses of CBD in vitro promoted a reduction (Dalterio et al., 1983).

The biphasic nature of CBD is observed in relation to the bioavailability of testosterone. As indicated above, CBD can act directly on testicular and hepatic microsomes of rodents, stimulating or inhibiting enzymes involved in testosterone metabolism and promoting the increase or reduction in androgen concentration. However, indirect actions through the HPG axis may also be involved.

Maternal treatment with a single dose of CBD during the perinatal period can alter endocrine function in adult male offspring when given at a certain gestational stage. In one study, although there were no changes in plasma and testicular concentrations of testosterone or FSH in adulthood in male mice prenatally exposed to CBD on day 12 of gestation (Dalterio & deRooij, 1986), in a previous study, male mice exposed on day 18 of gestation presented hormonal changes in adulthood (Dalterio, Steger, Mayfield, & Bartke, 1984a). In the study, half of the animals exposed to CBD in day 18 of gestation were castrated in adulthood, the other half remained intact. One week after castration, the animals received subcutaneous injections with testosterone and after 1 hour were submitted to blood collection by cardiac puncture for hormonal analysis. Intact or castrated mice showed no marked change in testosterone (plasmatic or testicular) and FSH concentrations. However, the concentrations of LH were normal in intact mice, but after testosterone injection in the castrated mice, LH concentrations increased. Increased LH following testosterone administration indicates a dysfunction in HPG axis integrity, particularly one that involves regulation of feedback mechanisms, which appears to be altered by perinatal exposure to CBD.

Male mice postnatally exposed on day 1 postpartum, by lactation, with CBD did not show changes in FSH plasma concentrations, while, LH concentrations were reduced (Dalterio & deRooij, 1986). Adult male mice offspring also postnatally exposed to CBD on day 1...
postpartum were separated into different groups for hormonal determination (Dalterio, Steger, Mayfield, & Bartke, 1984b). One group remained intact; another group received intratesticular injection of human chorionic gonadotropin (hCG) into one testis and 30 minutes later were castrated and the testis was homogenized for testosterone determination. To determine basal testosterone production in vitro, a third group underwent castration in which one of the testes was incubated for 4 h in the presence of hCG and the contralateral testis was incubated in the absence of hCG for the same period. This group still received a single subcutaneous dose of testosterone 1-week post-castration, and 1 hour later were subjected to cardiac puncture to estimate the concentrations of LH and FSH. Testicular testosterone concentration was reduced in intact animals and remained reduced in animals receiving intratesticular hCG. However, in vitro, after addition of hCG in the culture medium, there was an increase in testosterone concentration. LH was reduced in intact and post-castration mice and there was no effect on FSH.

These results again reveal a change in the feedback mechanism. Postnatal exposure to CBD showed reduced ability of the testes to respond in vivo to intratesticular injection of hCG in terms of increased testosterone production compared with baseline levels. It is possible that these changes in the feedback mechanism result from an interaction of CBD with testicular gonadotropin receptors, in the same way as that reported for androgen receptors (Purohit et al., 1980), influencing the mice’s endocrine function. It is important to emphasize that maternal treatment with CBD has had a long-term effect on the reproductive endocrine system of adult offspring. However, the effects were only evident when the pups were exposed from the 18th day of gestation, which allows us to propose that there is a certain period in the physiological development of the animals in which exposure to the CBD (placental or through lactation) is critical for the functioning of sex hormones.

Hormone concentrations after CBD exposure also were evaluated in male Rhesus monkeys (Rosenkrantz & Esber, 1980). The treatment was performed for 90 days and the effects were evaluated at the end of treatment and after 30 days of recovery. The authors reported a 30% reduction in testosterone at the end of treatment with the highest dosage, returning to normal after the recovery period. LH concentrations were elevated at higher doses after recovery, FSH increased after treatment and remained elevated at higher dosages after recovery. The fact that chronic treatment with CBD reduced testosterone concentrations in animals only during exposure suggests that these effects may be reversible. On the other hand, the concentrations of LH were increased after 30 days of recovery, suggesting a delayed response to the reduction in testosterone and evidence of interference in the feedback mechanism.

It is interesting to observe that, in most studies, plasma FSH concentrations were normal after CBD treatment despite significant changes in testosterone and LH concentrations. FSH concentrations were only altered after a long period of treatment with CBD. These results can be explained by the longer half-life of FSH in circulation with changes in FSH only becoming apparent sometime after the changes in LH and/or testosterone plasma levels (Steger et al., 1990). In addition, if FSH can induce the expression of LH receptors in interstitial testicular cells as it does in ovarian cells (White & Harrison, 2018), it is possible that high concentrations of this hormone are because of an attempt to increase the production of testosterone. However, indirect evidence of Sertoli cell dysfunction cannot be ruled out. Thus, it is possible that CBD acts directly on somatic cells, reducing concentrations of testicular inhibin and altering negative feedback between the testes and the pituitary. This may be relevant given the fact that Sertoli cells are essential for sperm production, and that changes in these cells may compromise spermatogenesis.

In recent years, there has been increased focus on the interaction between the production of sex hormones and the endocannabinoid system, as its constituents (receptors and enzymes) are located extensively in the structures of the HPG axis (Battista, Rapino, Di Tommaso, Bari, & Pasquariello, 2008; Carvalho, Santos, et al., 2018; Gye, Kang, & Kang, 2005). According to Gorzalka and Dang (2012), endocannabinoids (such as anandamide) act by centrally suppressing the release of gonadotrophin releasing hormone, LH and FSH by the hypothalamus and pituitary, as well as acting locally on the testes, altering the activity of Leydig and Sertoli cells (Figure 4). It has been reported that CBD increases the supply of anandamide and potentiates its action at binding sites (Carvalho, Santos, et al., 2018), as it is able to inhibit FAAH, the enzyme responsible for the degradation of this endocannabinoid (Leweke et al., 2012) (Figure 1). Thus, CBD may act through this endogenous system and promote the hormonal changes observed. Despite all these findings, the possible mechanisms by which CBD might influence reproductive endocrine functions are still under study.

### 3.3 Effects on reproductive organs

Treatment of rats and mice with CBD resulted in alterations in reproductive organ weights (Table 3). In rats exposed to CBD, reduced testis mass was recorded (Goldstein et al., 1977; Rosenkrantz & Hayden, 1979). CBD exposure was reported to promote reduction in the activity of esterase isoenzymes present in Leydig cells (Goldstein et al., 1977), which may have resulted in a reduction in testosterone level accompanied by testicular weight loss. It is known that the differentiation of sexual organs is regulated by testosterone action, showing the importance of this androgen in the maintenance of reproductive tissues, such as the testes (White & Harrison, 2018). On other hand, there were no changes in the mass of reproductive organs of mice receiving oral doses of CBD, neither three times a week for 5 weeks (Dalterio et al., 1982) and over periods of 15 and 35 days (Patra & Wadsworth, 1991), nor for 34 consecutive days (Carvalho, Santos, et al., 2018).

It must be considered that the pathway (oral) and pharmacokinetic factors such as metabolism rate, plasma distribution or tolerance development, due to differences between the species (rats and mice), may have influenced the result. However, the animals showed normality in hormonal concentrations, and most studies (Carvalho, Santos, et al., 2018; Dalterio et al., 1982) evaluated the effects in the period
after the end of the exposure (5–6 weeks), which may have provided enough time for a recovery in the tissue mass normality.

The effects of CBD oral administration in mice was also evaluated in the adult phase of the F1 generation. A single dose was administered in females on the 12th day of gestation or 12 hours after delivery, and the male offspring were evaluated during adulthood (Dalterio & deRooij, 1986). The authors reported a reduction in testis size of the prenatally exposed mice. On the other hand, administration on the 18th day of gestation resulted in an increase in the mass of the testes, and seminal vesicle in prenatally exposed pups (Dalterio et al., 1984a). The effects of postnatal exposure, in which female mice were treated with CBD on the day of delivery, were evaluated in male offspring during adulthood, which also resulted in a reduction in testis size (Dalterio et al., 1984b). These changes may be related to the period when CBD was administered, a period when the offspring’s sex organs were still immature.

The in vitro study concerning the vas deferens of mice showed that CBD increased the amplitude of ATP-mediated contractions and presented antagonistic action of the norepinephrine and cannabinoid on vas deferens receptor agonists (Pertwee, Ross, Craib, & Thomas, 2002). This tissue transports the spermatozoa through muscle contractions mediated mainly by the release of contractile neurotransmitters (ATP and norepinephrine). Norepinephrine is responsible for the (slow) tonic component of contraction, and ATP is responsible for the phasic component. Activation of the cannabinoid receptors in the vas deferens regulates the action of these neurotransmitters, inhibiting their release (Burnstock, 1988; Koslov & Andersson, 2013; Pertwee et al., 2002).

The high contractile response of the vas deferens to ATP may be the result of increased sensitivity to this neurotransmitter. However, this effect may have been the consequence of a reduced response to norepinephrine. Moreover, CBD also opposed the inhibitory activity of cannabinoid receptor agonists, which may have contributed to the amplitude of muscle contractions in response to ATP. However, the question of how CBD produces its various effects on the vas deferens remains to be resolved.

No general toxicity was correlated with the effects found in the reproductive tissues, as no death or significant changes in body weight of the animals have been reported. It is probable that the alterations are either indirect, because of disruption of pituitary-gonadal feedback regulation due to the influence of CBD on the endocannabinoid system (Gorzalka & Dang, 2012), or the direct result of CBD retention in the reproductive tissues and its subsequent direct action on them, as some changes occurred in the presence or absence of endocrine correlates. CBD inhibited the specific binding of dihydrotestosterone to the androgen receptor in the rat prostate (Purohit et al., 1980). This action shows the effect of CBD on androgen binding in the target tissues. Nonetheless, further studies are necessary to verify that these variations observed in the weight of testes and seminal vesicles in rats and mice exposed to CBD are related to functional alterations of tissue androgenic receptors. An increase or reduction in androgen sensitivity might be included among these functional alterations.

**FIGURE 4** Summary of the main interactions among the endocannabinoid system and the production of gonadotropins and androgens. (1) Endocannabinoids suppress the release of GnRH in the hypothalamus. (2) Reduction of GnRH, in turn, suppresses the release of LH and FSH in the adenohypophysis. (3) Reduced LH level. (4) Direct action of endocannabinoids on Leydig cells reduces the testosterone release. (5) Reduced FSH level. (6) Direct action of endocannabinoids on Sertoli cells. These endocrine actions promote impairment of spermatogenesis. FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone. Source: Gorzalka and Dang (2012).
3.4 | Effects on spermatogenesis

Studies with human and other mammal testes, shown in Table 4, were performed to evaluate dynamic spermatogenesis after exposure to CBD. These studies have shown alterations in testis cells (interstitial, somatic and germinative), which perform important functions during spermatogenesis. In relation to interstitial cells, in vivo treatment of mice with oral doses of CBD caused an increase in the nuclear area of Leydig cells, adjacent to the tubule in stage VII (Patra & Wadsworth, 1991). This result indicates that the interstitial cells either showed increased cellular activity or were injured by CBD action, which can be reflected in the synthesis of testicular steroids. Therefore, alterations in the synthesis of gonadal hormones may influence spermatogenic dynamics, as both sperm production and maturation are androgen-dependent processes (Courtois & Ortavant, 1981).

Studies with somatic testicular cells were also conducted to evaluate the effects of CBD (Table 4). In vitro, three CBD dosages contained in the culture medium with rat Sertoli cells were analyzed (Newton, Murphy, & Bartke, 1993). The results showed that the production of lactate by these cells was stimulated by higher doses of CBD. This effect could be related to the indirect action of CBD elevating serum FSH. However, changes in FSH appear to be evident only after a long period of exposure to CBD. Lactate is assumed to be released by the interstitial cells either showing changes in Sertoli cells 35 days after the end of oral treatment with CBD and/or from postpubertal patients with cryptorchidism. The authors evaluated the effects of CBD s (stage VII) suppressed the incorporation of [3H]-uridine by these cells (Tilk & Zimmerman, 1984). Uridine is a nucleoside used by germ cells in the metabolism of RNA, and its synthesis of this nucleic acid being more active in pachytenic spermatocytes and round spermatids (Geremia, Boitani, Conti, & Monesi, 1977; Monesi, Geremia, D’Agostino, & Boitani, 1978).

The reduction of uridine uptake served as an index of CBD interference, in vitro, in the macromolecular synthesis of spermatogenic cells. This result may explain the changes in the seminiferous tubules and germ cell maturation. In fact, Patra and Wadsworth (1991) reported modifications of these cells in mice treated with oral doses of CBD. Pyknosis was observed in pachytenic spermatocytes and a reduction in the amount of these cells after a 15-day treatment. At rest, spermatocytes were also reduced; however, after the 35-day treatment, the amount of these two cell types was normal. The authors observed the formation of multinucleated giant cells between the round spermatid layers and a reduction of spermatogonia type A in the mitotic stages (I, II, IX and XI) and spermatocytes II of the spermatogenic cycle. Several seminiferous tubules were shrunk and atrophied and showed vacuole formation in the tubule periphery. There was an increase in the lumen area, as well as desquamation and accumulation of germ cells inside the lumen.

In another in vivo study, alterations in the seminiferous tubules of mice, treated chronically with CBD, were also shown (Carvalho, Santos, et al., 2018). Therein that study, an increase in the mitotic stages (I-VI) and a reduction in spermiation (VII-VIII) and meiotic stages (XII) were observed. However, the authors reported that the seminiferous tubules in the sperm stages showed an increase in diameter and circumference, as well as in the height of the seminiferous epithelium, indicating an increase of germ cells at this stage. As the animals in this study were killed at postnatal day 90, i.e., 35 days after completion of treatment, it may be proposed that the reduction of germ cells induced by CBD may be reversible. It is interesting to observe that Leydig cells adjacent to the tubule in stage VII had nuclear hypertrophy, probably due to an increase in metabolic activity (Patra & Wadsworth, 1991). The metabolic activity of Sertoli cells was also stimulated in the presence of CBD (Newton et al., 1993). It is reasonable to hypothesize that the observed increase of germ cells in the sperm stage was related to these events. On the other hand, the postnatal exposure of mice to CBD on day 1 postpartum, via the milk from the lactating dams to their pups, promoted reduction in the quantity of elongated spermatids of the adult pups.

It is not possible to say whether this result was due to a direct action in these cells (through apoptosis) or in precursor cells (with impairment in the process of cell division and/or differentiation), but the effect of long-term CBD on germ cells may contradict the hypothesis of reversibility. However, two factors should be considered: the period in which the animals were exposed to the CBD and...
the period in which they were submitted to being killed for histological evaluations. Exposure during gonadal development may be responsible for the reduction of elongated spermatids in adult offspring. Moreover, as the killing of the mice occurred before postnatal day 90, meaning there were only a few spermatogenic cycles (França & Russell, 1998; Hess, Schaeffer, Eroschenko, & Keen, 1990), it is probable that the cellular recovery process was not detected at that time.

Together, the results of these studies point to CBD having a gonadotoxic action in mammalian spermatogenesis, mediated by several mechanisms. Among them, the interaction with the endocannabinoid system giving rise to cytotoxicity in testicular cells stands out. Differentiation of sperm cells is a process involving the joint action of functional, somatic and interstitial cells. It is not yet clear whether alterations in spermatogenesis reflect the direct action of CBD on the seminiferous epithelium or are secondary to an alteration in the hormonal milieu of the testes.

Indeed, the changes reported in Leydig cells can compromise the proper supply of androgens in the testicular microenvironment. In addition, another question to consider involves the metabolic activity of Sertoli cells. Just as lactate production was stimulated by the action of CBD, it is possible that production of the androgen-binding protein by Sertoli cells was stimulated too. Alterations in the production of this protein can locally decrease the proportion of free hormone available for the maintenance of spermatogenesis (Hansson et al., 1976). However, all the hypotheses presented here are only speculations, and future studies are indispensable to clarify these questions.

3.5 | Effects on sexual behavior, sperm morphology and fertility

CBD has been reported to affect sexual behavior, sperm morphology and the fertility of vertebrates and invertebrates (Table 5). CBD interference on the sexual behavior of chronically exposed mice was recently evidenced (Carvalho, Souza, et al., 2018). A reduction (with a higher dose) or an increase (with a lower dose) in latencies of first mount, intromission and ejaculation was reported. These parameters are common measures of sexual performance in male mice, where the increase represents difficulty in performing copulation and the reduction represents a facilitation of sexual behavior, as they characterize the growing sexual interest of the male mouse by the receptive female and a diminished ejaculatory threshold (Canseco-Alba & Rodríguez-Manzo, 2016). The lower dose reduced the amount of ejaculations during behavioral evaluation. It is known that in the CNS, activation of the endocannabinoid system inhibits, whereas activation of TRPV1 receptors stimulates, the sexual behavior in rodents (Gorzalka, Hill, & Chang, 2010). In view of this, a biphasic nature of CBD action on copulation was suggested. Thus, lower doses of CBD may indirectly increase the concentration of endocannabinoids, mainly by inhibiting FAAH and increasing the supply of anandamide at the cannabinoid receptors, while higher doses of CBD could block these receptors, or activate TRPV1 receptors, facilitating sexual behavior.

The authors also emphasized the importance of emotional influence, demonstrating that the animals may have presented anxiety-type responses, given the new evaluation environment (Carvalho, Souza, et al., 2018). Thus, the higher dose of CBD may have exhibited anxiolytic properties and have contributed to the improved sexual performance of the animals and the lower dose may have generated an anxiogenic response and hindered sexual behavior. However, the authors highlighted the importance of future neurobehavioral studies to clarify the exact mechanisms by which CBD acts on copulation.

In vivo morphological evaluations of mouse sperm cells showed different results. There was no change in the sperm morphology of the cauda of the epididymis following acute treatment with CBD (Zimmerman, Bruce, & Zimmerman, 1979). On the other hand, data from the assessment of sperm cells of mice present in the epididymal cauda (Patra & Wadsworth, 1991) and in the vas deferens (Carvalho, Santos, et al., 2018) after chronic treatment with CBD, revealed abnormalities in the head and tail morphology of spermatozoa.

Morphological changes in the sperm cells of mice coincided with the effects of CBD on spermatogenesis reported by these authors, which presented a series of alterations in the cells of the seminiferous and interstitial epithelium. However, as suggested by Carvalho, Santos, et al. (2018), CBD may have a direct action on the spermatozoa of mice through interaction with the endocannabinoid system, as receptors and enzymes of the endocannabinoid system have already been identified in human spermatozoa (Agirregoiti et al., 2010; Aquila et al., 2009; Francavilla et al., 2009). Treatment of sea urchin (an invertebrate model) spermatozoa in culture medium containing CBD also revealed head abnormalities in these cells, which had electrodense lipid deposits in the subacrosomal and centriolar region (Chang & Schuel, 1991; Schuel, Chang, et al., 1991). These effects of CBD on the morphology of sperm can compromise the fertilization of the ovum and result in male infertility.

In fact, CBD exposure has been reported to interfere in fertilization in invertebrate and vertebrate males. In vitro studies with sea urchin spermatozoa (Chang & Schuel, 1991; Schuel, Chang, et al., 1991; Schuel, Berkery, et al., 1991; Schuel, Schuel, Zimmerman, & Zimmerman, 1987) showed that the doses used of CBD inhibited the ability of spermatozoa to fertilize sea urchin eggs, and that this inhibition was caused by a blockade of the spermatic acrosome reaction (the final stage of sperm capacity for later interaction with the zona pellucida and the oocyte membrane).

Although several factors, such as biochemical, morphological or endocrine changes, may affect the fertilization capacity of spermatozoa, the presence of lipid deposits in the subacrosomal region of sea urchin sperm has led to the assumption that CBD may promote the activation of phospholipases within the sperm cell, thus releasing free arachidonic acid from membrane phospholipids. Arachidonic acid, in turn, would prematurely trigger the acrosome reaction or inhibit it, reducing the fertilizing capacity of sea urchin spermatozoa. In vivo treatments of male mice with repeated doses of CBD also exhibited changes in male fertility. Male mice treated for 5 weeks (3 days/week) presented a reduction in fertility rate and an increase in prenatal and postnatal deaths of pups (Dalterio et al., 1982). The treatment of male
mice for 34 consecutive days showed a reduction in the number of fertilized females and in the number of pups generated (Carvalho, Souza, et al., 2018).

Perinatal treatment with CBD of female mice, exposed prenatally (on day 12 of gestation) and postnatally (on day 1 postpartum), resulted in a reduction in the number of live pups by adult male offspring, while a reduction in fertilization rate was observed only in adult male offspring exposed postnatally (Dalterio & deRooij, 1986). Certainly, disturbance in the fertility of mice can be present in the apparent absence of endocrine dysfunction, as most of these authors reported that CBD treatment did not promote alteration of the gonadal hormones. In addition, there is evidence that cannabinoids and TRPV1 receptors, located in human spermatozoa, are crucial elements in the regulation of sperm function in the female genital tract (Bernabò et al., 2012; Maccarrone et al., 2005).

In this context, it has been proposed that CBD can prematurely activate the acrosome reaction in mice through enzymatic inhibition and accumulation of anandamide in CB1 receptors or through the reaction with TRPV1 receptors (Carvalho, Souza, et al., 2018). It is interesting to note that anandamide is synthesized from membrane phospholipids, which corresponds to the reports of Schuel et al. (1987) and Schuel, Chang, et al. (1991) on the action of these compounds on the fertilizing capacity of sea urchin spermatozoa. The results obtained after CBD exposure indicate that, in addition to the observed effects on sexual behavior and on sperm morphology, effects on the fertility of vertebrates and invertebrates are also apparent. In mice, functional deficits in fertility were observed, including a reduction in the number of live offspring and in the rate of successful fertilization. Although a reduction in fertility may not be dramatic in this very prolific species, these findings may have more serious implications for the CBD-exposed human population, particularly in those individuals in which fertility deficits may already exist.

4 | CONCLUSIONS

In this review, we analyzed the pharmacological basis of CBD, focusing on its involvement at the central and peripheral levels in the male reproductive system. From the results of the studies identified, it is possible to conclude that CBD has several harmful effects on this system. These include impairment on sexual behavior, reduced testosterone levels, testicular cell degeneration and decreased fertilization rates. In addition, studies indicate that effects on reproductive function can be seen in vitro and in vivo, with acute or chronic exposures in mammalian vertebrates and invertebrates. However, knowledge is still limited, and additional research is required to elucidate fully the mechanisms of action, as well as the reversibility of CBD effects on the reproductive system.

These future investigations are important because of the widespread use of cannabis for recreational purposes besides the advancement in evidence of the therapeutic potential of CBD. In this respect, the effect of CBD on male reproduction, summarized in this review, is an important subject, given the fact that cannabis is widely used by young people who are in the process of sexual maturation or who are at a stage in their lives when they are considering having children. In addition, this review demonstrates the importance of the rational use of CBD as a therapeutic substance, preserving reproductive safety. Thus, it is important to clarify and to alert patients about the risk-benefit relationship of CBD treatment during the prescription of this cannabinoid.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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