Effects of Cannabidiol on the Male Reproductive System

Renata Mazaro e Costa
Federal University of Goiás (Brazil)
mazaro@ufg.br
The presenter declares that there exists no real or perceived conflict of interest.
Male Reproductive Health Is Neglected

Sperm quality and concentration are reduced

(15 \times 10^6/ml; Vit.: 58%; Mot.: 32%; Morf.: 4.0% - WHO, 2021)
In Brazil, in 2014, CBD was approved by the Federal Council of Medicine for the treatment of children and adolescents with refractory epilepsy.

Upon learning about the presence of the endocannabinoid system in the male reproductive tract, this approval brought us a question: could the use of CBD during such an important period for sexual maturation be harmful?
Cannabidiol—Action Mechanism

1. Anandamide synthesis
2. Inhibitory action of CBD
3. High levels of AEA in the endocannabinoid system
4. Gi protein activation resulting in inhibition of Ca^{2+} input and hyperpolarization by intracellular K^{+} output
5. Activation of MAPK activity
6. Activation of TRPV1 receptor and depolarization by voltage-dependent Ca^{2+} channels opening
7. Change in gene expression
8. Activation of adenylate cyclase and decrease of cAMP and PKA activity

“The primary outcomes associated with the most adverse events reported in the literature were neurological (13) and developmental and reproductive (12).

“…brings to light the need for a well-designed, guideline-compliant reproductive toxicity study with CBD. …can serve as a crucial starting point from which to conduct additional analyses, such as systematic reviews on key subtopics of interest (e.g., the potential toxicity of CBD exposure to the male reproductive system).
Cannabidiol—Reproductive Studies

- Rats (44%)
- Mice (38%)
- Men (3%)
- Monkeys (3%)

Vertebrates (88%)

- Sea urchin

Invertebrates (12%)

in vivo (50%)

in vitro (50%)

Model

Method

Time

Acute (61%)

Sub-acute (26%)

Carvalho, RK; Andersen ML; Mazaro-Costa, R. The effects of cannabidiol on male reproductive system: a literature review. Journal of Applied Toxicology, 2018.
Cannabidiol—Hepatic Testosterone Metabolism

C_{19}H_{28}O_{2}

2-α-hydroxytestosterone
Narimatsu et al., 1990

6-β-hydroxytestosterone
Bornheim & Correia, 1989

7-α-hydroxytestosterone
List et al., 1977

16-α-hydroxytestosterone
Narimatsu et al., 1990, Bornheim & Correia, 1989

Androstenedion
Narimatsu et al., 1990

(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
HPT: Hypothalamus-Pituitary-Testis axis

Cannabidiol—17α-hydroxylase Enzyme

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 interstices of the mature testes. Goldstein et al., 1977

Cannabidiol—HPT Axis (Mouse and Rats)

Testosterone
Reduced binding DHT to receptor
(Harclerode et al., 1979; Jakubovic et al., 1979; Purohit et al., 1980; Rosenkrantz & Esber, 1980; Dalterio et al., 1984; Carvalho et al., 2018)

No effect on Testosterone, Progesterone, Estradiol, LH and FSH.
(Dalterio et al., 1982; Steger et al., 1990; Carvalho et al., 2022; Henderson et al., 2023)

LH (Dalterio & deRooij, 1986)
FSH
Testosterone (Dalterio et al., 1983)
Cannabidiol—Sexual Behavior

Table 1
Sexual behavior of male Swiss mice treated orally for 34 days with CBD at doses of 15 and 30 mg/kg (CBD 15 and CBD 30 groups, respectively) and Control group, after a recovery period of 20–30 days, during a 30-minute observation period.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>GROUPS</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 8)</td>
<td>CBD 15 (n = 9)</td>
<td>CBD 30 (n = 10)</td>
</tr>
<tr>
<td>First mount latency (min)</td>
<td>1.8 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 ± 5.5&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.3 ± 1.0&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>First intromission latency (min)</td>
<td>5.1 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.9 ± 5.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1 ± 1.5&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>First ejaculation latency (min)</td>
<td>8.9 ± 6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3 ± 8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9 ± 8.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of mounts prior to the first ejaculation</td>
<td>9.0 ± 5.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0 ± 10.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of intromissions prior to the first ejaculation</td>
<td>4.0 ± 2.0</td>
<td>3.0 ± 4.0</td>
<td>5.0 ± 7.0</td>
</tr>
<tr>
<td>Postejaculatory mount latency (min)</td>
<td>1.9 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0 ± 3.8&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Postejaculatory intromission latency (min)</td>
<td>1.7 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1 ± 4.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 ± 1.9&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total number of mounts</td>
<td>31.0 ± 15.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.0 ± 13.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.0 ± 16.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total number of intromissions</td>
<td>12.0 ± 6.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.0 ± 5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.0 ± 15.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total number of ejaculations</td>
<td>4.0 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Sperm count and daily sperm production (DSP) on testis of 90-day-old Swiss mice orally treated for 34 days with 15 and 30 mg kg⁻¹ body weight of CBD 15 and 30 groups, respectively, and control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 10)</td>
</tr>
<tr>
<td>Mature spermatids (x10⁶ per testis)</td>
<td>16.9 ± 1.0</td>
</tr>
<tr>
<td>Relative mature spermatids (x10⁶ g⁻¹ testis)</td>
<td>134.0 ± 9.8</td>
</tr>
<tr>
<td>DSP (x10⁶ testis day⁻¹)</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>Relative DSP (x10⁶ testis g⁻¹ day⁻¹)</td>
<td>27.5 ± 2.1</td>
</tr>
</tbody>
</table>


Sperm count of adult Swiss mice from control group and groups treated for 34 days with cannabidiol (CBD) at a dose of 15 and 30 mg/kg bw. Control group received sunflower oil. Values expressed as mean ± SEM, p > 0.05 to ANOVA.

# Cannabidiol—Fertility

Table 2

Fertility of male Swiss mice treated orally for 34 days with CBD at doses of 15 and 30 mg/kg (CBD 15 and CBD 30 groups, respectively) and Control group, after a recovery period of 5–15 days.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Control (n = 9)</th>
<th>CBD 15 (n = 10)</th>
<th>CBD 30 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertility rate (%)</td>
<td>90.00 ± 31.62(^a)</td>
<td>100.00 ± 0.00(^a)</td>
<td>70.00 ± 48.30(^\text{b})</td>
</tr>
<tr>
<td>Gestational weight gain (g)</td>
<td>26.71 ± 5.03</td>
<td>26.87 ± 4.63</td>
<td>23.36 ± 3.92</td>
</tr>
<tr>
<td>Number of offspring</td>
<td>13.00 ± 1.60(^a)</td>
<td>12.00 ± 1.72(^a)</td>
<td>10.00 ± 1.63(^\text{b})</td>
</tr>
<tr>
<td>Male (%)</td>
<td>50.74 ± 12.62</td>
<td>49.45 ± 19.24</td>
<td>57.22 ± 14.94</td>
</tr>
<tr>
<td>Female (%)</td>
<td>49.26 ± 12.70</td>
<td>50.55 ± 19.24</td>
<td>42.78 ± 14.94</td>
</tr>
<tr>
<td>Offspring weight (g)</td>
<td>1.15 ± 0.05</td>
<td>1.08 ± 0.04</td>
<td>1.14 ± 0.03</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>0.11 ± 0.00</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Corpora lutea number</td>
<td>15.00 ± 2.30</td>
<td>14.00 ± 2.13</td>
<td>14.00 ± 2.23</td>
</tr>
<tr>
<td>Implantation number</td>
<td>14.00 ± 1.62</td>
<td>13.00 ± 2.00</td>
<td>12.00 ± 2.56</td>
</tr>
<tr>
<td>Resorption number</td>
<td>1.00 ± 1.07</td>
<td>1.00 ± 0.79</td>
<td>2.00 ± 3.04</td>
</tr>
<tr>
<td>Pre-implantation loss (%)</td>
<td>6.00 ± 6.27</td>
<td>6.00 ± 5.58</td>
<td>10.00 ± 7.67</td>
</tr>
<tr>
<td>Post-implantation loss (%)</td>
<td>7.00 ± 6.71</td>
<td>6.00 ± 5.50</td>
<td>16.00 ± 18.90</td>
</tr>
</tbody>
</table>
Cannabidiol—Spermatogenesis and Testicular Cells

- Promoted degeneration of seminiferous tubule and inhibition of sperm maturation. (Rosenkrantz & Hayden, 1979).
- Elongated spermatid was reduced in rats treated on day 1 post-partum. (Dalterio & deRooij, 1986)
- Leydig cell: nuclear area was greater (Patra & Wadsworth, 1991)
- Tubular cells reduced to a single layer - enlarged lumen
- Vacuole formation at the periphery of the tubules
- Desquamation and accumulation of germ cells within the lumen of the tubules;
- Several tubules were shrunken and atrophied (Patra & Wadsworth, 1991)
Cannabidiol and Spermatogenesis

Type A spermatogonia: reduced in stages I, II, VII, IX and XI; Pachytene spermatocytes: pyknosis among cells (15 days).

Cell types were normal after 35 days, but round spermatid: multinuclear giant cell formation within the nest of the spermatids (Patra & Wadsworth, 1991)
Table 2
Frequency of germinal epithelium stages obtained from cross-sections of seminiferous tubules of male Swiss mice orally treated for 34 days with 15 and 30 mg/kg body weight cannabidiol (CBD 15 and CBD 30 groups, respectively) and control group.

<table>
<thead>
<tr>
<th>Germinall epithelium stage</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>I-IV</td>
<td>29.0 ± 3.0</td>
</tr>
<tr>
<td>V-VI</td>
<td>19.0 ± 5.0</td>
</tr>
<tr>
<td>VII-VIII</td>
<td>23.0 ± 3.0</td>
</tr>
<tr>
<td>IX</td>
<td>11.0 ± 4.0</td>
</tr>
<tr>
<td>X-XI</td>
<td>16.0 ± 5.0</td>
</tr>
<tr>
<td>XII</td>
<td>8.0 ± 1.0</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation. ANOVA followed by Tukey’s post-hoc test (*p < 0.001).

Carvalho et al. Decreasing sperm quality in mice subjected to chronic cannabidiol exposure: new insights of cannabidiol-mediated male reproductive toxicity. *Chemico-Biological Interactions*, 2022
Sertoli Cells

**Table 3**

Number of Sertoli cells per section of seminiferous tubules at stages I–IV, V–VI, VII–VIII, and XII of 90-day-old Swiss mice orally treated for 34 days with 15 and 30 mg kg⁻¹ body weight of CBD 15 and 30 groups, respectively, and control group.

<table>
<thead>
<tr>
<th>Germinal epithelium stage</th>
<th>Sertoli cells (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 10)</td>
</tr>
<tr>
<td>I–IV</td>
<td>116.2 ± 7.1ᵃ</td>
</tr>
<tr>
<td>V–VI</td>
<td>117.0 ± 3.0ᵃ</td>
</tr>
<tr>
<td>VII–VIII</td>
<td>119.3 ± 4.8ᵃ</td>
</tr>
<tr>
<td>XII</td>
<td>131.7 ± 6.1ᵃ</td>
</tr>
<tr>
<td>Total</td>
<td>484.2 ± 12.6ᵃ</td>
</tr>
</tbody>
</table>

Cannabidiol and Testicular Cells—Sertoli

Sertoli cells \((in \ vitrō\) model - human cell)  
- Inhibition of the G1/S-phase cell cycle transition and cell proliferation and DNA synthesis;  
- Decreased cell viability;  
- Downregulated key cell cycle proteins;  
- Reduced the mRNA and protein levels.

- Activates p53 (which can upregulate p21 signaling pathways) and upregulates p16 (key regulator of senescence), leading to cellular senescence.

Cannabidiol—Testicular Cells (Sertoli)

Sertoli cells (human and mouse *in vitro* model)

- Inhibited cell growth by inducing a G1 arrest in cell cycle progression;
- Inhibited DNA synthesis at the S phase;
- Altered the F-actin organization;
- Induced apoptosis (at 25 - 30 µM).

7-carboxy-CBD (less cytotoxic) and 7-hydroxy-CBD: inhibited cellular proliferation and decreased DNA synthesis

Cannabidiol—Testicular Cells (Leydig)

Leydig cells (human *in vitro* model)

- Inhibited cell growth by inducing a G1/S arrest in cell cycle progression;
- Decrease DNA synthesis;
- Induced apoptosis (+caspase-3/7).

7-carboxy-CBD (lowest cytotoxicity) and 7-hydroxy-CBD: decrease cell viability and induce apoptosis

Sperm morphology (approximately 200 per animal) in vas deferens and presence/position of the cytoplasmic droplet in 90-day-old Swiss mice orally treated for 34 days with 15 and 30 mg kg\(^{-1}\) body weight of CBD 15 and 30 groups, respectively, and control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control((n = 10))</th>
<th>CBD 15((n = 10))</th>
<th>CBD 30((n = 10))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>102.0 ± 26.0</td>
<td>100.0 ± 15.0</td>
<td>95.0 ± 10.0</td>
</tr>
<tr>
<td>Head abnormalities</td>
<td>49.0 ± 18.0(^{a})</td>
<td>60.0 ± 14.0(^{b})</td>
<td>57.0 ± 16.0(^{b})</td>
</tr>
<tr>
<td>Tail abnormalities</td>
<td>35.0 ± 18.0</td>
<td>36.0 ± 9.0</td>
<td>37.0 ± 18.0</td>
</tr>
<tr>
<td>Isolated head</td>
<td>14.0 ± 18.0(^{a})</td>
<td>4.0 ± 4.0(^{b})</td>
<td>11.0 ± 10.0(^{a})</td>
</tr>
<tr>
<td>Cytoplasmic droplet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>91.0 ± 35.0(^{a})</td>
<td>67.0 ± 20.0(^{b})</td>
<td>70.0 ± 21.0(^{b})</td>
</tr>
<tr>
<td>Proximal</td>
<td>1.0 ± 1.0(^{a})</td>
<td>1.0 ± 1.0(^{a})</td>
<td>0.0 ± 0.0(^{b})</td>
</tr>
<tr>
<td>Medial</td>
<td>104.0 ± 33.0(^{a})</td>
<td>127.0 ± 22.0(^{b})</td>
<td>125.0 ± 22.0(^{b})</td>
</tr>
<tr>
<td>Distal</td>
<td>4.0 ± 4.0</td>
<td>5.0 ± 5.0</td>
<td>5.0 ± 5.0</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation \((P < .05)\). Generalized linear model (Poisson regression, identity) \(\(^{a}\)P < .001)\).

\(^{a}\)Values similar to the control group.

\(^{b}\)Significant differences between treated and control groups.

Sperm morphology in the vas deferens of CBD 15 (n=10) and CBD 30 (n=10) group, observed using light microscopy. (A) Normal sperm; (B) isolated head (arrow); (C–E) head abnormalities: (C) sperm with “banana” head (arrow); (D) sperm with round head; (E) sperm with flattened head; (F–I) tail abnormalities, sperm with folded tail: (F) proximal region; (G) distal region; (H) medial region and presence of head with no hook (arrow); (I) sperm with coiled tail; (J) sperm with medial cytoplasmic droplet (arrow)

Motility of sperm from cauda epididymis. (A) percentage of motile spermatozoa; (B) kinematic parameters: curvilinear velocity (VCL), average pathway velocity (VAP), straight-line velocity (VSL). Frequency of sperm with acrosome-intact, reacted and abnormal of adult Swiss mice from control group and groups treated for 34 days with cannabidiol (CBD) at a dose of 15 and 30 mg/kg bw (B). Control group received sunflower oil. Values expressed as mean ± SEM. ANOVA followed by Tukey's post-hoc test (*p<0.05; **p<0.01).

Schematic drawing of the action of CBD 30 mg/kg on mouse spermatozoa.
(a) Localization of endocannabinoid system elements in the mouse sperm cell, adapted from Rapino et al (2014); (b) premature activation of the acrosome reaction during transit in the male reproductive tract of Swiss mice treated with CBD at a dose of 30 mg/kg; 1) inhibition of FAAH by CBD; 2) increase of anandamide in CB1 receptor; 3) modification of the activation dynamics of TRPV1 receptors in sperm head; 4) opening of calcium channels before TRPV1 migration to the acrosomal region, initiating the influx of Ca2+ in the sperm head before entering the female tract and impairing fertility. Abbreviations: NAPE-PLD, N-arachidonoylphosphatidylethanolamine-specific phospholipase D; FAAH, fatty acid amide hydrolase; CB1/2, type-1 or 2 cannabinoid receptor; TRPV1, transient receptor potential vanilloid type 1; AEA, N-arachidonoylethanolamine or anandamide

Representative comet assay image under a fluorescence microscope at 200× magnification showing sperm cells (stained using SYBR Green I stain) and the percent of DNA in the comet tail (A) and leukocytes cells (B). DNA damage expressed as % tail DNA in sperm cells of male Swiss mice from control group and group treated for 34 days with cannabidiol (CBD) at 15 and 30 mg/kg bw (B). Control group received sunflower oil. Values expressed as mean ± standard error of mean. ANOVA followed by Tukey’s post-hoc test (*p < 0.001).

Carvalho et al. Decreasing sperm quality in mice subjected to chronic cannabidiol exposure: new insights of cannabidiol-mediated male reproductive toxicity. Chemico-Biological Interactions, 2022
In vivo effect of cannabidiol (CBD) on antioxidant enzymes activities of sperm from cauda epididymis of adult Swiss mice from control group and groups treated for 34 days with CBD at doses of 15 and 30 mg/kg bw. The control group received sunflower oil. (A) SOD activity; (B) CAT activity, MDA. Values expressed as mean ± standard error of mean. ANOVA followed by Tukey's post-hoc test (*p<0.05; **p<0.001). SOD: Superoxide dismutase; CAT: catalase; MDA: malondialdehyde.

Carvalho et al. Decreasing sperm quality in mice subjected to chronic cannabidiol exposure: new insights of cannabidiol-mediated male reproductive toxicity. *Chemico-Biological Interactions*, 2022
NOAELs for CBD isolate were identified in rats (OECD 421 study).

- 100 mg/kg-bw/d: F0 male and female systemic toxicity and female reproductive toxicity;
- 300 mg/kg-bw/d: F0 male reproductive toxicity;
- 100 mg/kg-bw/d: F1 neonatal and F1 generation toxicity.

NOAEL—No Observed Adverse Effect Level

The need for further studies to attest to the safety and effectiveness of CBD use, especially in the long term.

Studies related to reproduction are scarce, especially regarding prolonged use.

CBD action mechanism remains under study, and its effects on reproduction are not fully known.

A growing use of CBD by men in reproductive age alerts us.
Acknowledgements

SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety
Acknowledgements

Special Thanks for collaborators:

Prof. Dr. Francisco Guimarães—FMRP/USP
Prof. Dr. Monica Levy Andersen—UNIFESP
Prof. Dr. Daniela Mello—DGen—ICB/UFG
Prof. Dr. Fernanda Alcântara—DHisto—ICB/UFG
Prof. Dr. Paulo Ghedini—DFar—ICB/UFG
Prof. Thiago Rocha—IPTSP—UFG

Dr. Bruno Gonçalves
Dr. Hericles Campos
Dr. Fábio Fernandes—UNESP
Come to Goiânia - the Art Déco capital in Brazil.