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Cannabis and male reproduction: Impact on offspring via sperm epigenome

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Abstract: Cannabis is the most widely used drug in the Western societies particularly among adolescent and young adults. Epidemiological studies demonstrate that men use cannabis more frequently than women with higher risk in developing cannabis-related disorders. Although the direct adverse effects of cannabis on male reproductive functions have been studied both in humans and animal models, the possible long-term risks for the health of the users and their offspring are still an area of active research.

Introduction

Cannabis is the most widely used illicit drug in Western societies. In the last decade, with expanding cannabis legalization, its use strongly increased for medical and recreational purposes. It is estimated that 3% of the world's population consumes marijuana with an increase in the number of US cannabis users by 60% between 2007 and 2017, mainly among males of reproductive age (World Drug Report, 2019). This trend is associated with a decrease in the perception of risk of harm of cannabis products and with an increase of its safety (Jordan *et al.*, 2020). In this context, a change in the cannabis market has developed, leading to the availability of new forms of cannabis with a high content of tetrahydrocannabinol (THC), the main psychoactive component. In the early 1990s, the average THC content in marijuana was less than 4% while in 2018 it was more than 15% (ElSohly *et al.*, 2016; Stuyt, 2018). Although THC is the best-known psychoactive constituent of the plant, cannabis also contains more than 500 other chemicals, including more than 100 cannabinoids that are chemically related to THC but whose properties are still unknow (Amin and Ali, 2019). Higher potency strains of cannabis are associated with increased risk of developing a cannabis use disorder like psychosis and addiction (Blanco *et al.*, 2016) and with a rise of concerns on the potential reproductive consequences, particularly in young males that are the most common users. Surprisingly, coincident are the observations of worldwide decline of semen quality in developed countries and delayed conception for many couples compared to previous generations (Mann *et al.*, 2020). Therefore, a possible link between use of cannabis and decline in sperm parameters and quality needs to be elucidated. Here, the current knowledges related to the adverse effects of THC/cannabinoids on male reproductive health are discussed. Special attention is paid to epigenetic modifications induced by the drug and potential risks of defects in the offspring transmitted by the sperm.

Effect of cannabis on sperm parameters

Both THC and cannabinoids exert their effects by binding to cannabinoid receptor type 1 (CB_1) and/or type 2 (CB_2) G protein-coupled receptors, which are also the receptors of endogenous endocannabinoids (eCBs) like anandamide (AEA) and 2-arachidonoylglycerol (2-AG). All together cannabinoid receptors, eCBs and all the enzymes for the biosynthesis and degradation of eCBs, form the endocannabinoid system (ECS). The ECS is deeply involved in a broad array of biological functions and any interference with this system, through exogenous cannabinoids exposure, can alter the balance of downstream endogenous signaling pathways with possible pathological consequences (Maccarrone *et al.*, 2015).

In male, the ECS is a key player in several reproductive processes both in humans and animals. In mouse, ECS is present and active in male germ cells and controls at least two critical steps of the germ cell differentiation: 1) the transition from mitotic to meiotic stage, during which testicular endocannabinoid 2-AG, produced by spermatogonia, might act as an autocrine factor via CB_2 receptors promoting meiotic entry (Grimaldi *et al.*, 2009); 2) spermiogenesis, during which AEA, produced by Sertoli cells and/or by spermatids, might

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act as a paracrine/autocrine factor via CB_1 receptors to regulate sperm morphogenesis (Migliaccio *et al.*, 2018). Since a similar expression pattern of many components of the ECS has been recently described in human male germ cells, ECS might play a similar role in human spermatogenesis (Nielsen *et al.*, 2019). Accordingly, exposure to exogenous cannabinoid compounds that bind to CB_1 and CB_2 receptors could alter these critical steps of spermatogenesis, leading to altered sperm functions.

Several studies reported that cannabis consumption affects spermatogenesis and sperm functions, including sperm number, morphology, motility and lastly sperm epigenome (Grimaldi *et al.*, 2013; Rossato *et al.*, 2005; Barchi *et al.*, 2020; Szutorisz and Hurd, 2016; Meccariello *et al.*, 2020; Innocenzi *et al.*, 2019). This last point is of particular significance since sperm epigenome modifications in cannabis consumers could have potential long-term effects on offspring health.

Both animal and *in vitro* studies reported a negative impact of cannabis (THC/cannabinoids) on male fertility with a reduction in sperm count and motility and with altered morphology (Murphy *et al.*, 2018; Innocenzi *et al.*, 2019; Whan *et al.*, 2006; Verhaeghe *et al.*, 2020). In humans, a few studies assessed the effects of cannabis smoking on male reproductive functions, reporting conflicting results. Gundersen *et al.* (2015) found that healthy young men who regularly smoked marijuana more than once per week had significantly lower sperm count than those who did not smoke marijuana or those who used the drug less frequently (Gundersen *et al.*, 2015). In contrast, recently Nassan *et al.* (2019) reported that men that have smoked marijuana at some point in their life had significantly higher concentrations of sperm when compared with men who have never smoked marijuana. One possible hypothesis has been suggested: a moderate use of marijuana may have a pro-spermatogenic effect improving testicular function while at higher dose, this relation reverses, resulting in adverse effects (Nassan *et al.*, 2019). However, this aspect, in humans, remains a matter of debate and requires further investigation.

Effect of cannabis on sperm epigenome

One of the most exciting area of research is the effect of cannabinoid/THC on sperm epigenome that aims to identify the epigenetic modifications induced by the drug in the sperm (Schrott and Murphy, 2020). The establishment and maintenance of the paternal epigenetic program is important for appropriate gamete function and quality and alterations of sperm epigenome may influence the health and development of next generations. Epigenetic mechanisms include DNA methylation at CG dinucleotides, post-translational modifications of histones tails, and noncoding RNA (ncRNA). These modifications regulate gene expression without altering the DNA sequence and are important modulator of cell differentiation, including spermatogenesis. In animal models several evidence showed that paternal exposure to THC/cannabinoid has effects on health of next generation, causing behavioural and neurobiological abnormalities in the offspring. However, to date, only few evidence in animal models have linked epigenetic modifications identified in the offspring with those present in the sperm of THC/cannabinoids exposed animals.

In a study, Murphy *et al.* (2018) reported that cannabis use in humans and THC exposure in rats, is associated with widespread changes of sperm DNA methylation (Murphy *et al.*, 2018). In a follow up study, they found that sperm of rat exposed to Cannabis showed hypomethylation in the autism candidate gene *Dlgap2* and that the same hypomethylated state of this gene was present in the nucleus accumbens of rat brain born from THC exposed fathers, strongly supporting the potential inheritance of an altered sperm DNA methylation pattern (Schrott *et al.*, 2019). Besides *Dlgap2*, the same group identified other genes involved in neurodevelopment that are differentially methylated in sperm of THC exposed rats respect to control unexposed rats, suggesting that could be inherited by the offspring determining aberrant expression in the brain (Levin *et al.*, 2019; Schrott *et al.*, 2020).

In a study performed by our group, the effect of the synthetic cannabinoid JWH-133, selective agonist of CB_2 receptor, on mouse spermatogenesis and sperm quality has been investigated (Grimaldi *et al.*, 2009; di Giacomo *et al.*, 2016). The interest towards this receptor is based on its high level of expression in spermatogonia. In *in vitro* experiments, JWH-133 treatment of spermatogonia induced changes in histone methylation levels of H3K4m3 and H3K9m2 in genomic flanking regions of meiotic genes *c-Kit* and *Stra8*, increasing their expression and promoting meiotic entry of these cells (di Giacomo *et al.*, 2016). As consequence, JWH-133 chronic exposure of immature male mice showed accelerated spermatogenesis onset and reduced spermatozoa number recovered by epididymis, pointing out the importance of correct endocannabinoid signaling for proper spermatogenesis (di Giacomo *et al.*, 2016). Furthermore, in a follow up study we reported that sperm from JWH-133 treated mice maintained the ability to fertilize egg, but embryo growth and placenta development were impaired. One possible explanation is that *in vivo* over-activation of this receptor, through JWH-133 exposure of male mice, could determine epigenetic aberrations in germ cells that are then paternally inherited by the offspring through the sperm. We demonstrated that DNA methylation of paternally expressed imprinted genes *Peg10* and *Plagl1*, two genes important for placental development, resulted hypermethylated in sperm of JWH-133 exposed male and the same DNA modification was retained in the placenta and associated with reduced placenta development and embryo growth in the progeny from JWH-133 exposed father (Innocenzi *et al.*, 2019). These is the first evidence of epigenetic changes induced by THC/cannabinoids in germ cells and transmitted to the new individual by sperm. A proposed model is reported in Fig. 1.

Conclusions and perspectives

In the recent years increasing evidence indicate that father's lifetime experiences, such as cannabis use, can be transmitted to the offspring, affecting health and development. Thus, it is important to know the potential consequences of cannabis exposure not only in the father but also in the offspring. Recent studies suggested that

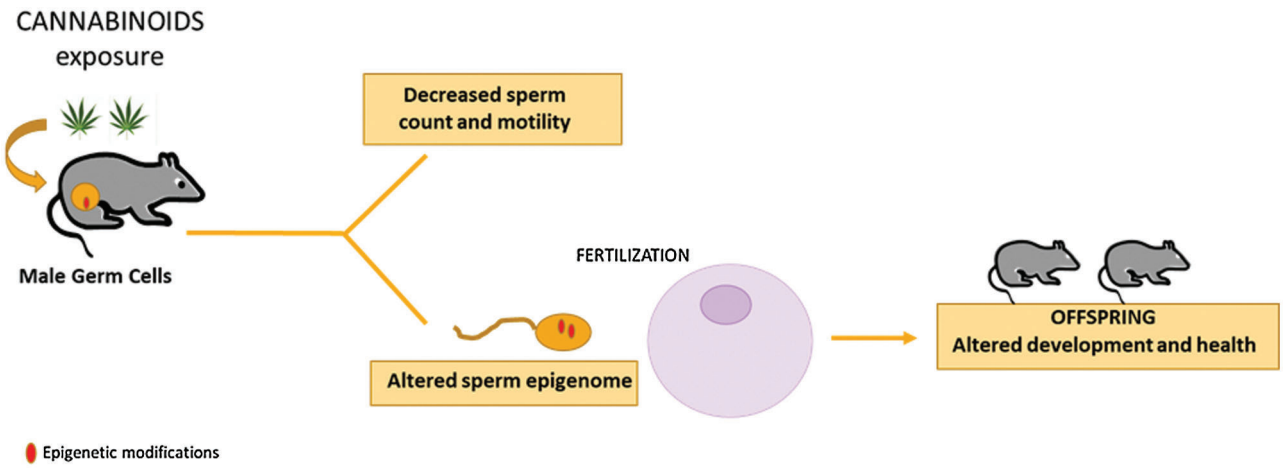

FIGURE 1. Cannabinoid exposure on male reproduction. Cannabinoid exposure affects male reproduction by impairing sperm number and motility in the exposed animal and by altering development and health of the offspring via sperm epigenome.

epigenetic alterations in the sperm could be involved in the transmission of cannabis-induced traits from father to offspring. Therefore, more attention needs to be paid to fathers' lifestyle and on the potential effects on sperm epigenome. Prevention should encourage men to live healthy lives and should increase risks perception of cannabis consumption to protect the health of users and their offspring.

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