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Effects of substance exposure on gametes and pre-implantation embryos: a narrative review

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Review Article

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Abstract

Substance use refers to the consumption of drugs that have varying degrees of impact on a persons' physical, mental and emotional well-being. While the adverse health effects of drugs have been extensively documented, further research is needed to understand their impact on fertility. Studies have indicated that substance use affects both the male and female reproductive systems. As substance use is more prevalent among young adults compared with the elderly, it appears that individuals of reproductive age are particularly vulnerable to the reproductive impairments associated with substance use. Although numerous studies have reported detrimental effects of substance use on pregnant women and their foetus during the post-implantation stages, there are limited studies on critical pre-implantation period and gamete stages. In this narrative review, we aimed to focus on the most significant evidence regarding the impact of substances on gametes and pre-implantation embryos.

Introduction

Substances (drugs) are any psychoactive compounds that have the ability to cause harm to the health of the individual or society. These substances can be divided into different groups according to their pharmacological properties and behavioural effects, including nicotine, alcohol, cannabinoids, opioids, depressants, stimulants and hallucinogens (McLellan, 2017). Drug abuse refers to the habitual use of addictive drugs to alter one's mood (Holbrook & Rayburn, 2014). Nicotine is an addictive compound found in tobacco products. In recent years, new devices such as electronic cigarettes have entered the market, causing a shift in peoples' exposure to nicotine compared with the past (Price & Martinez, 2019; Yingst *et al.*, 2019). Despite the notable harm of alcohol consumption on health, alcohol addiction is prevalent among people around the world (Graham *et al.*, 2011; Labhart *et al.*, 2017). Cannabinoids are commonly used in two forms, marijuana and hashish. Although both marijuana and hashish are natural and have plant origins, a large number of synthetic cannabinoids are also available in the market. The legalization of marijuana consumption in some countries has led to an increase in the number of consumers. The main psychoactive compound in cannabis is delta-9-tetrahydrocannabinol (Δ9-THC) (Panlilio *et al.*, 2015). Opioids include components such as heroin, buprenorphine, codeine, tramadol and oxycodone, which were initially introduced into the market for pain relief. Unfortunately, their unnecessary and none-medical use led to problems such as abuse, dependence, addiction and drug overdose deaths around the world (Butler & Stechlinski, 2023; McLellan, 2017; Roussin *et al.*, 2022; Zhao *et al.*, 2019). Depressant drugs that slow down the nervous system are prescribing for the improving insomnia in people. Compounds such as benzodiazepines and barbiturates fall into this category (McLellan, 2017; Petrushevska & Velik Stefanovska, 2015). Stimulants include compounds such as cocaine, amphetamine and methamphetamine (MAMP). These compounds facilitate and increase the production of certain neurotransmitters in the nervous system while having high potential for creating dependence and addiction (Ciccarone, 2011). The use of hallucinogens such as LSD, mescaline and MDMA is increasing among young people, and over time, they cause changes in the behaviour and brain of users (Wu *et al.*, 2008).

According to the United Nations Office on Drugs and Crime (UNODC) report, drug use is more prevalent among young adults than older individuals, with some exceptions regarding traditional drug use (Drugs & Crime, 2018; Phillips *et al.*, 2017). Both men and women engage in substance abuse. Although the prevalence of substance use is higher in men, there are increasing concerns regarding the medical, psychiatric and social consequences of substance abuse in women compared with men (McHugh *et al.*, 2014). A great number of women who use drugs are

in their childbearing years, resulting in a higher number of pregnant women with addiction issues (Haight *et al.*, 2018). It is important to note that any illicit drugs that are not protein-bound can freely cross the blood-placental barrier and affect the embryos and foetus (Ganapathy, 2011; Holbrook & Rayburn, 2014). Some women may continue drug use until they receive confirmation of their pregnancy, which means that the embryos of such women are exposed to addictive substances during critical stages of embryogenesis (Holbrook & Rayburn, 2014).

Reproductive-aged adults appear to be more susceptible to impairments caused by drug abuse. Studies have reported that drug abuse affects the male and female reproductive systems (Barazani *et al.*, 2014; Hill *et al.*, 2005; Hugues *et al.*, 1980; Ragni *et al.*, 1988). Previous research has investigated the effects of drug use on pregnant women and their embryo (or foetus) during the post-implantation stages (Blandthorn *et al.*, 2018; Corsi *et al.*, 2020; Prasad & Jones, 2019; Shah *et al.*, 2022). However, there are limited studies on the effects of drug abuse during critical pre-implantation period and gamete stages. The present study aims to focus more on these subjects.

Effects of substance exposure on oocytes

Several studies have provided evidence for the presence of different opioid receptors in human oocytes. Delta, kappa and mu have been shown to be expressed in human oocytes. Kappa receptors are detected in the peripheral region of germinal vesicle (GV) oocytes. During oocyte maturation, the distribution pattern changes to a more internal location at metaphase I (MI) and becomes homogeneous at metaphase II (MII). Mu receptors also change their distribution pattern from the margin of the GV oocyte to all regions of MI and MII oocytes. Delta receptors, on the other hand, are located in the peripheral region of oocytes and do not change during oocyte maturation (Agirregoitia *et al.*, 2012). Similar distribution patterns have been observed for cannabinoid-degrading enzymes and cannabinoid receptor 1 (CB1) in human oocytes. Fatty acid amide hydrolase (FAAH), a cannabinoid-degrading enzyme, is located at the margin of GV and MI oocytes, similar to CB1 receptors. During oocyte maturation, FAAH spreads throughout all parts of MII oocytes. Monoglyceride lipase (MGLL), another cannabinoid-degrading enzyme, does not change during oocyte maturation (Agirregoitia *et al.*, 2016). Based on these distribution patterns, Agirregoitia *et al.* concluded that opioids and cannabinoid system may play a role in oocyte maturation (Agirregoitia *et al.*, 2012, 2016). Cannabinoid receptors have been found to be expressed in various parts of the female reproductive system, including the ovaries, oviduct and uterine endometrium (Bari *et al.*, 2011; Walker *et al.*, 2019). Endogenous opioids (enkephalins and endorphins) are small molecules that serve as hormones and neuromodulators in the central nervous system (CNS) and exert various physiological effects in the body and also reproductive system (Böttcher *et al.*, 2017; Faden, 1984). Enkephalins are produced in corpus luteum and have a role in precise functions of the reproductive system. In the mouse, the presence of embryo stimulates the production of enkephalins, which in turn it helps in the transport of the embryo in the uterine tube. However, enkephalins can have other functions; for example, they may play a role in the physiology of granulosa cell or they may alter movement of fetal intestine (Buéno *et al.*, 1986; Cupo *et al.*, 1987).

Dell'Aquila et al. conducted a study on bovine cumulus-oocyte complexes (COCs) and mural granulosa cells and found that they have mRNA coding for the μ-opioid receptor. The supplementation of *in vitro* maturation (IVM) medium with hormone and β-endorphin did not result in any differences in the rates of oocytes reaching the MII stage compared with the control group. However, GV oocytes exposed to IVM hormone-free medium supplemented with β-endorphin showed a decreased rate of maturation. The inhibitory effect of β-endorphin was reversed by Naloxone. The authors reported that the mu-opioid receptor affects oocyte maturation by inducing an increase in intracellular calcium levels (Dell'Aquila *et al.*, 2002). Additionally, the expression of CB1, FAAH and MGLL in human granulosa cells suggests a potential role of this system in the nuclear maturation of oocytes (Agirregoitia *et al.*, 2015). In a mouse model, CB1 activation during oocyte IVM was found to modulate Akt and ERK1/2 phosphorylation status and improve embryo production. In the absence of CB1, *in vivo* maturation of oocytes reduced, and embryo development was delayed (López-Cardona *et al.*, 2017). Increased histone acetylation, decreased histone methylation and changes in expression of non-coding RNA(s) are some epigenetic alterations in brains of opioid users (Browne *et al.*, 2020). Therefore, considering the presence of different drug receptors on oocytes and different parts of the female reproductive system, drug exposure during critical stages of oogenesis and embryogenesis can impact oocyte and embryo development.

Δ9-THC can affect the follicular phase of the menstrual cycle. Acute administration of Δ9-THC leads to a reduction in FSH levels. Consequently, follicle development, oocyte maturation and steroid production in the ovary are impaired, resulting in a lack of ovulation in the menstrual cycle and ultimately infertility (Brents, 2016). Misner et al. investigated the effects of THC on oocyte maturation and embryo development. Immature bovine oocytes were cultured with THC for 24 h during IVM. THC significantly decreased the rate of oocyte maturation to the MII stage and subsequently reduced the cleavage rate on day 2 post-fertilization. No differences in spindle morphology were observed in the matured oocytes. Furthermore, there was no significant difference in the rate of development or the proportion of trophectoderm to inner cell mass cells at the blastocyst stage between the treatment and control groups. However, the level of apoptosis in these blastocysts increased at doses of 0.32 and 3.2 μM THC (Misner *et al.*, 2021).

In a study conducted by Nematollahi Mahani et al, it was observed that morphine administration increased the number of atretic follicles in mouse ovaries and affected folliculogenesis. However, there were no significant differences in the volume and weight of the ovaries between the control and addicted groups (Nematollahi Mahani *et al.*, 2005). Similarly, the administration of cocaine in rhesus monkeys, as noted by Chen et al, disrupted the normal pulsatile secretion of gonadotropins and altered mean E2 levels during the late follicular phase (Chen *et al.*, 1998). Additionally, Potter et al. found that low-dose follicular-phase cocaine administration impaired menstrual cyclicity and folliculogenesis (Potter *et al.*, 1999).

It has been shown that MAMP administration leads to an increase in the number of fragmented oocytes and a decrease in oocyte quality and embryo development (Nezhad *et al.*, 2016). Another study conducted by Wang et al, investigated the long-term exposure of adolescent mice to MAMP and its effects on ovarian reserve. They found that intraperitoneal injections of MAMP at a dose of 5 mg/kg (three times per week) for 8 weeks impaired ovarian reserve. The treated mice exhibited a decrease in the number of primordial and growing follicles and an increase in

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atretic follicles. Furthermore, there was a decrease in the secretion of anti-Mullerian hormone (AMH), oestradiol and progesterone in granulosa cells. The ovaries treated with MAMP also showed mitochondrial swelling and degeneration in the granulosa cells, potentially leading to apoptosis in the ovarian tissue (Wang *et al*., 2016).

According to the available literature, tobacco smoke has the ability to interfere with the normal progression of folliculogenesis and development. This disruption can cause heightened levels of apoptosis or autophagy, DNA damage and abnormal connections between oocytes (immature eggs) and granulosa cells (supporting cells), ultimately resulting in the demise of ovarian follicles. In addition, epigenetic alterations including down-regulation of antioxidant genes (Gpx1 and Wnt10b) and the steroid biosynthesis gene (Fdx1) in ovarian tissue as well as down-regulation of Gja1, Lama1 and the Ferroptosis indicator (Gpx4) in granulosa cells were reported following CS exposure (Li *et al*., 2022). Furthermore, there is evidence suggesting the presence of persistent oxidative stress in ovarian tissue exposed to tobacco smoke. This oxidative stress is characterized by notable increases in the levels of reactive oxygen species (ROS) within the mitochondria, lipid peroxidation and activity of the CYP2E1 detoxification enzyme. These detrimental effects contribute to a reduction in the potential for successful fertilization and can lead to overall dysfunction of the oocytes (Sobinoff *et al*., 2013). The administration of high concentrations of nicotine (≥0.5 mM) to oocytes has been found to have a significant impact on their maturation. This high nicotine concentration leads to notable changes in the subsequent process of meiosis, resulting in abnormal configurations of chromosomes within the oocytes (Racowsky *et al*., 1989).

Cigarette smoking can induce oxidative stress in granulosa cells, DNA damage in cumulus cells surrounding the oocytes, and increase thickness of the zona pellucida in women undergoing the assisted reproductive technology (ART) (Budani *et al*., 2017; Shiloh *et al*., 2004; Sinkó *et al*., 2005). Furthermore, exposure to 1-(N-methyl-N-nitrosamino)-1-(3-pyridinyl)-4-butanal, a residual compound of cigarette and a secondary pollutant, has been reported to have detrimental effects on oocytes including increased DNA damage, impaired spindle morphology, epigenetic alterations and apoptosis (Liu *et al*., 2019). Additionally, cigarette smoke exposure during pregnancy has been associated with alterations in offspring oocyte quality and histone methylation, which can affect the proper functioning of genes involved in oocyte maturation and development (Gao *et al*., 2017).

There is conflicting evidence about the effect of alcohol on female infertility. Although some studies reported no association between alcohol consumption and menopausal age (Dorjgochoo *et al*., 2008; Kaczmarek, 2007), there are also other studies demonstrating that alcohol consumption tends to be correlated with postmenopausal status (Brett & Cooper, 2003; Cooper *et al*., 2001; Freeman *et al*., 2021).

Nardo et al. demonstrated that there is no significant difference in plasma AMH concentrations and antral follicle count (AFC) between women's alcohol consumers and non-consumers enrolled in ART (Nardo *et al*., 2007). Ozbakir & Tulay showed that the number of antral follicles was very similar in women with or without alcohol consumption. Although the number of oocytes and metaphase II (MII) oocytes tended to be higher in the control group, there was no significant difference (Ozbakir & Tulay, 2021). Ultrastructure of ovarian tissue from rats exposed to ethanol administration revealed the harmful effects of ethanol in the primordial and secondary follicles, oocytes, granulosa cells, corona radiata cells and zona pellucida (Faut *et al*., 2009).

Firns et al. demonstrated that there is no significant association between women's alcohol consumption and fertility parameters including number of retrieved oocytes, fertilization rate, β-hCG pregnancy rate or pregnancy loss (Firns *et al*., 2015). The nc886 gene, a non-coding RNA transcribed via RNA polymerase III, is methylated in the oocyte and silenced on the maternal allele in nearly 75% of humans. Carpenter et al. showed that oocyte age and preconceptual alcohol consumption are associated with epigenetic imprinting of nc886 (Carpenter *et al*., 2021).

It is important to note that there is a significant gap in the literature regarding the effects of addictive drugs on oogenesis and oocyte quality. This highlights the need for further studies, particularly at the molecular level. Table 1 indicates a summary of substance effects on oocytes and oogenesis.

Effects of substance exposure on sperm cells

The investigation of the effects of opioids on sperm cell motility and morphology highlights the importance of opioid receptors and endogenous opioid peptides in the modulation of reproduction in mammals. Albrizio et al. used western blot/indirect immunofluorescence to demonstrate the expression of delta opioid receptors on equine spermatozoa and its relationship with sperm cell physiology. The study utilized double CTC/Hoechst staining to evaluate viability, capacitation and acrosome reaction, while mitochondrial activity was assessed using MitoTracker Orange dye. The localization of the delta opioid receptor was observed in the sperm tail mid-piece as a doublet of 65 and 50 kDa molecular mass. Furthermore, the delta opioid receptor antagonist, naltrindole, was found to modulate various physiological parameters of equine spermatozoa, including motility, capacitation, acrosome reaction and viability in a dose-dependent manner (Albrizio *et al*., 2010).

Another study investigated the impact of opiates on human semen quality, antioxidant capacity of seminal plasma, function and DNA integrity of spermatozoa. The results showed that opiate consumption led to a decrease in sperm concentration, catalase-like and superoxide dismutase-like activity. Additionally, sperm DNA fragmentation was found to significantly increase in opiate consumers (Safarinejad *et al*., 2013).

Azari et al. evaluated the effects of different doses of tramadol (10 mg/kg and 20 mg/kg) on sperm parameters and testicular tissue in mice. Tramadol was injected intraperitoneally three times per week for six weeks. The results indicated that tramadol administration led to a decrease in sperm concentration, motility and vitality. Tramadol also affected the germinal layer and some seminiferous tubules, resulting in decreased spermatogenesis in the germinal epithelium of affected seminiferous tubules. More degenerative modifications were observed with a dose of 20 mg/kg tramadol at week six. However, most histopathological changes returned to the normal structure by week 12 (Azari *et al*., 2014). One study reported reduced sperm counts, sperm vitality and free testosterone levels in patients with tramadol abuse. Higher levels of prolactin and abnormal sperm morphology were observed in these patients (Bassiony *et al*., 2020).

López-Cardona et al. evaluated whether THC can affect the ability of spermatozoa to fertilize and produce embryos in a mice model of chronic THC treatment (10 mg/kg/day THC for 30 days). Although the expression of CB1 significantly reduced in the

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Table 1. Summary of the substance's effects on oocyte

Substances		Species	Results	Reference
Opioids	β-endorphin	Bovine	- The presence of the mu-opioid receptor gene in the cumulus-oocyte complex (COC). - No effect of β-endorphin on the rates of maturation to metaphase II.	(Dell'Aquila *et al.*, 2002)
	Morphine	Mouse	- Increased number of atretic follicles in the ovary. - Moderate alterations in folliculogenesis.	(Nematollahi Mahani *et al.*, 2005)
Cannabinoids	Δ9-THC	Bovine	- Decreased rate of oocyte maturation to metaphase II and subsequently the rate of cleavage at day 2 post-fertilization. - No significant difference in spindle morphology in maturated oocytes. - No significant difference in the rate of development or the proportion of trophectoderm to inner cell mass cells at the blastocyst.	(Misner *et al.*, 2021)
Stimulants	Cocaine	Rhesus monkey	Suppression of the normal increase in FSH and LH pulse amplitude observed in the late follicular phase (cycle days 11 to 15).	(Chen *et al.*, 1998)
			Disruption of menstrual cyclicity and folliculogenesis.	(Potter *et al.*, 1999)
	MAMP	Mouse	- Decreased number of primordial and growing follicles. - Increased number of atretic follicles. - Decreased secretion of anti-Mullerian hormone (AMH), oestradiol, and progesterone in granulosa cells. - Mitochondrial morphological damage and activation of apoptosis in the ovarian tissue.	(Wang *et al.*, 2016)
			- Increased number of fragmented oocytes - Decreased oocyte quality	(Nezhad et al., 2016)
Nicotine	Nicotine	Human	Increased risk of idiopathic POF	(Chang *et al.*, 2007)
			- Decrease in antral follicle count - Increase in serum follicle-stimulating hormone (FSH) levels - No significant difference in LH and oestradiol levels	(Caserta *et al.*, 2013)
			- Decreased ovarian response to hyperstimulation - Lower clinical pregnancy rate - Lower serum AMH concentrations	(Freour *et al.*, 2008)
			- Increased oxidative stress - Decreased fertilization rate - Decreased implantation rate - Decreased pregnancy rate - No difference in number of aspirated metaphase II oocytes, embryos quality and live birth rate.	(Budani *et al.*, 2017)
			Increased DNA damage in human cumulus cells	(Sinkó *et al.*, 2005)
			Increased thickness of zona pellucida	(Shiloh *et al.*, 2004)
		Mouse	- Down-regulation of antioxidant genes (Gpx1 and Wnt10b) and the steroid biosynthesis gene (Fdx1) occurred in ovary - Increased oxidative stress - Increased DNA damage and cellular aging - Inhibition of KGN cell proliferation by inducing G1-phase cell cycle arrest - Down-regulation of Gja1, Lama1 and the Ferroptosis indicator (Gpx4) in granulosa cells	(Li *et al.*, 2022)
			- Decreased of the number of oocytes - Decreased of the zona pellucida oocytes diameter - Increased of thickness of zona pellucida - Decreased of the oocyte's nucleus diameter - Alterations of the histone methylation in germinal vesicle oocytes	(Gao *et al.*, 2017)
			- Increase ROS production - Increased lipid peroxidation - Increased activity of the CYP2E1 detoxification enzyme - Decreased primordial and antral follicles - Increased time to be pregnant	(Sobinoff *et al.*, 2013)
			- Disruption of meiotic spindle morphology - Inhibition of ERK1/2 activation - Decreased cleavage and blastocyst rate - Epigenetic alterations including altering DNA and histone methylations by reducing 5 mC and H3K4me2 levels	(Liu *et al.*, 2019)

(*Continued*)

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Table 1. (*Continued*)

Substances	Species	Results	Reference
		- Increased apoptosis - Oxidative stress	
	Hamster	Increased disturbances in oocyte meiotic maturation	(Racowsky *et al.*, 1989)
Alcohol	Human	No significant difference in AMH and AFC	(Nardo *et al.*, 2007)
		No significant difference in AFC and number of metaphase II (MII) oocytes	(Ozbakir & Tulay, 2021)
		No significant association between women's alcohol consumption and fertility parameters including number of oocytes, fertilization rate, pregnancy rate or loss of pregnancy	(Firns *et al.*, 2015)
Alcohol		Decreased of number of eggs retrieved following ART	(Klonoff-Cohen *et al.*, 2003)
		Significant fewer oocytes retrieval following ART	(Rossi *et al.*, 2011)
		Association with epigenetic imprinting of nc886	(Carpenter *et al.*, 2021)
	Rat	- Significant reduction in the number of primordial follicles - Higher frequency of preantral and atretic follicles	(Chuffa *et al.*, 2009)
		Negative effects on secondary follicles, oocyte, granulosa cells, corona radiata cells, and zona pellucida	(Faut *et al.*, 2009)

THC-mice cortex, CB1 mRNA was not affected in the testis. Moreover, no alterations were observed in testes histology, sperm motility or concentration. No changes were observed in the methylation of evaluated three CpG regions of CB1 in the embryos produced via *in vitro* fertilization (IVF) (López-Cardona *et al.*, 2018). In a bovine model, THC affected motility, morphology, capacitation and mitochondrial potential of spermatozoa and also disrupted the expression of key microRNAs associated with early embryonic development (Favetta, 2023).

Several studies have explored the direct effects of heroin on male infertility. For instance, Nazmara et al. conducted research on men who had used heroin for at least one year to investigate its effects on semen quality. The study found that sperm motility and viability were significantly lower in the addicted group compared with the control group. Additionally, semen pH and sperm histone replacement abnormalities were significantly higher in the addicted group. However, no significant difference was found regarding serum sex hormones between the two groups. These findings suggest that the decreased quality of sperm in heroin users may be attributed to both the direct effects of heroin on opioid receptors in sperms and its indirect effects, such as increased ROS levels (Nazmara *et al.*, 2019).

The activity of aminopeptidase N (APN), an essential metalloenzyme, was found to be altered and potentially contribute to male subfertility by affecting spermatogenesis, motility and viability of spermatozoa (Irazusta *et al.*, 2004). A case-controlled study investigated the correlation between the expression of APN/CD13 and NEP/CD10 genes and semen quality in heroin-addicted men and fertile men. The study found a significant decrease in sperm progressive motility, total motility and viability in the heroin-addicted group compared with normozoospermic (normal sperm) men. Moreover, the expression levels of APN and NEP genes were lower in heroin users compared with normozoospermic men. These findings suggest a significant association between heroin addiction, asthenozoospermia (reduced sperm motility), and reduced mRNA expression levels of APN and NEP. Additionally, the duration of drug dependence was found to be associated with sperm motility and viability, as well as gene expression levels of NEP and APN. Heroin may directly decrease progressive and total sperm motility due to modification in the enkephalin-degrading enzymes (Rezaei-Mojaz *et al.*, 2020). In a study carried out by Gornalusse et al., the impact of heroin consumption on an important epigenetic mechanism was examined. The researchers detected an altered cargo of small RNAs (sRNAs) in human spermatozoa, which was associated with chronic heroin consumption (Gornalusse *et al.*, 2023). Furthermore, heroin users were reported to experience leukocytospermia (elevated white blood cell count in semen), asthenozoospermia, increased DNA fragmentation and epigenetic alterations (Nazmara *et al.*, 2021).

Ebrahim et al. investigated the ultrastructural morphology of spermatozoa in diacetylmorphine-addicted patients. The findings revealed that diacetylmorphine consumption can impact the histone-to-protamine ratio, motility, viability and morphology of spermatozoa (Ebrahim *et al.*, 2020).

One study examined the impact of codeine, the most commonly abused opioid, on sperm quality in New Zealand white rabbits. The results demonstrated that codeine treatment significantly reduced sperm membrane integrity and various sperm parameters, including normal morphology, viability, count and motility. Additionally, codeine treatment led to increased oxidative damage, caspase 3 activity and sperm DNA fragmentation. The study concluded that chronic use of codeine primarily affects sperm quality and DNA fragmentation through oxidative stress (Ajayi & Akhigbe, 2020). In another study, *in vitro* effects of different concentrations of codeine (0, 0.1, 1, 5 and 10 mM) were assessed on human spermatozoa. The findings revealed that all tested concentrations of codeine significantly decreased sperm motility and plasma membrane integrity. Additionally, the level of sperm 8-hydroxy-2-deoxyguanosine (8-OHdG), an indicator of oxidative DNA damage, increased in a time-dependent manner (Akhigbe *et al.*, 2021).

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Regarding marijuana use, significant differences in the sperm concentration and total sperm count were detected in men who had ever used marijuana compared with those who had never used it. However, no significant differences were observed in the sperm concentration between current and past marijuana smokers. Men who had ever smoked marijuana had lower sperm motility and follicle-stimulating hormone (FSH) concentrations than non-smokers, but there was no association between marijuana smoking and other reproductive hormones or sperm DNA integrity markers (Nassan *et al.*, 2019). On the other hand, Gundersen et al. reported that regular marijuana smoking (more than once per week) was associated with decreased sperm concentration and total sperm count. They also found that marijuana smokers had higher levels of testosterone compared with non-smokers (Gundersen *et al.*, 2015). Furthermore, marijuana consumption was found to negatively affect sperm motility and morphology (Carroll *et al.*, 2020).

In studies involving methamphetamine (MAMP), it was found that administration of MAMP for 7 or 14 days in rats had adverse effects on testes structure and spermatogenesis. The number of seminiferous tubule cells decreased significantly, as did the number of spermatogonia, primary and secondary spermatocytes. Moreover, various spermatogenesis indices, including the mean seminiferous tubule diameter, tubular differentiation index, repopulation index and spermiogenesis index, significantly reduced in testicular tissue (Saberi *et al.*, 2017). Another study investigated the effects of MAMP on proliferation and apoptosis in the rat seminiferous tubules. The treatment resulted in decreased cellular proliferation and the proliferation/apoptosis index ratio. The staining of rat testis with a marker of proliferation (PCNA) showed a 75% decrease in PCNA-positive spermatogonia due to MAMP administration. TUNEL results indicated an increase in TUNEL-positive spermatogonia in some seminiferous tubules. Furthermore, gaps were observed in the epithelium between the layer of spermatogonia and other layers of cells in MAMP-treated rats (Alavi *et al.*, 2008). Yamamoto et al. evaluated the induction of apoptosis in mouse seminiferous tubules by administering MAMP at different doses (1, 5, 10 and 15 mg/kg). The findings revealed that MAMP, in doses above 5 mg/kg, induced apoptosis in spermatogenic cells. Additionally, a dose of 15 mg/kg inhibited male copulatory behaviour by reducing serum testosterone levels (Yamamoto *et al.*, 2002). The effect of MAMP on neurotransmitter secretion, such as serotonin, may be one of causes of cell apoptosis or proliferation (Alavi *et al.*, 2008; Kalant, 2001). It has also been reported that (MDMA) ecstasy can reduce the levels of Gonadotropin-releasing hormone (GnRH) and serum testosterone by affecting the hypothalamic-pituitary-testicular axis (Dickerson *et al.*, 2008; Fronczak *et al.*, 2012).

Many studies conducted in recent decades have shown effects of tobacco consumption on semen and sperm parameters. Accordingly, it has been shown some alterations in sperm morphology, as well as decreases in the sperm concentration, motility and viability among individuals who smoke (Asare-Anane *et al.*, 2016; Dai *et al.*, 2015; Künzle *et al.*, 2003). Additionally, tobacco smoking was found to decrease the levels of zinc and Ca+2 ATPase in seminal plasma, leading to reduced sperm motility (Kumosani *et al.*, 2008). Individuals who smoke heavily are at a higher risk of experiencing ultrastructural abnormalities in sperm, such as alterations in axonemal microtubules and tails, which can have a detrimental impact on sperm motility (Yeung *et al.*, 2009; Zavos *et al.*, 1998). Nicotine consumption through smoking also hampers the acrosome reaction and capacitation (Shrivastava *et al.*, 2014; Zalata *et al.*, 2004). Consequently, smoking has been linked to compromised sperm maturation, reduced sperm function and diminished fertilization potential of sperm (Dai *et al.*, 2015; Harlev *et al.*, 2015). Besides, animal studies have shown that nicotine induces reduction in the number of germ cells, Leydig cells, and Sertoli cells, and potentially causing male infertility (Ahmadnia *et al.*, 2007; Kim *et al.*, 2005; La Maestra *et al.*, 2015). Nicotine also affects the activity of testicular androgenic enzymes and plasma testosterone level. This ultimately disrupts the process of spermatogenesis and reduces fertility (Jana *et al.*, 2010).

Tobacco smoke has been associated to cause with not only decreased semen quality, but also abnormal protein expression, genetic and epigenetic abnormalities in sperm (Linschooten *et al.*, 2013; Marchetti *et al.*, 2011; Pereira *et al.*, 2014). Both animal and human studies have shown genome instability, genetic mutations and the presence of aneuploids in the germline of individuals exposed to tobacco smoke (Beal *et al.*, 2017; Hassold *et al.*, 1996; Omolaoye *et al.*, 2022; Pereira *et al.*, 2014). The process of protamination, which is crucial for fertility, is likewise affected by cigarette smoking due to the impact of various chemicals present in tobacco smoke on chromatin structure (Hamad *et al.*, 2014). Additionally, smoking has been linked to decreased activity of sperm glutathione peroxidase (GPx-1, 4) and reduced mRNA expression of glutathione reductase in spermatozoa (Viloria *et al.*, 2010). One study found that there is a distinct difference in gene expression at the mRNA and miRNA levels in the spermatozoa of men who smoke (Marchetti *et al.*, 2011). Another study revealed that tobacco smoke leads to specific changes in the miRNA content of spermatozoa in smoking men. These miRNA alterations are believed to play a role in the regulatory pathways crucial for maintaining healthy sperm and normal embryo development (Marczylo *et al.*, 2012). Additionally, Chen et al. demonstrated that exposure to tobacco smoke for six weeks resulted in changes in the expression of sperm proteins in mice. The affected proteins were associated with energy metabolism, reproduction and the development of structural molecules (Chen *et al.*, 2015). Elevated levels of ROS following smoking can disturb male fertility via damage to sperm DNA, lipid peroxidation and impaired spermatogenesis (Calogero *et al.*, 2023; Kumar *et al.*, 2015; Sansone *et al.*, 2018; Wright *et al.*, 2014).

Effects of alcohol abuse in sperm cells have been investigated in several studies. Accordingly, it has been shown that habitual alcohol consumption caused lower quality of semen and alterations in reproductive hormones (Jensen *et al.*, 2014). Lwow and colleagues evaluated the effect of occasional alcohol consumption on semen quality and reported no impact on semen quality. However, the percentage of macrocephalic sperm cells increased significantly in consumers (Lwow *et al.*, 2017). In the other study, it has been shown that alcohol consumption can decrease semen quality and also increase ROS production and DNA damage (Finelli *et al.*, 2021; Kotova *et al.*, 2013). Rompala et al. evaluated the effect of heavy chronic intermittent ethanol consumption on sperm cells in mice and found alterations in none-coding small RNAs, including tRNA-derived small RNA, mitochondrial small RNA and microRNA. In this way, alcohol abuse may induce epigenetic alterations in offspring (Rompala *et al.*, 2018). In another study in mice, paternal heavy chronic alcohol consumption in periconceptional period caused foetal growth restriction via alterations in sperm inherited non-coding RNA(s) (Bedi *et al.*, 2019).

Epigenetic alterations have been observed in sperm from male drug addicts, indicating changes in gene expression, protamine

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deficiency, and alterations in the miRNAs and non-coding RNAs (Chorbov *et al.*, 2011; Hamad *et al.*, 2014; Marczylo *et al.*, 2012; Nazmara *et al.*, 2020; Nazmara *et al.*, 2021; Rompala *et al.*, 2018). These studies have demonstrated significant detrimental effects of drugs on various sperm parameters, as well as epigenetic status and sexual function. However, it is important to note that larger-scale clinical trials are recommended to provide more robust conclusions, particularly when considering other factors such as lifestyle patterns that may influence the generalizability of these findings (Srinivasan *et al.*, 2021). Table 2 indicates an overview of the effects of substances on sperm, summarizing the observed impacts on sperm parameters and reproductive health.

Effects of substance exposure on pre-implantation embryos

The expression of opioid receptors at various stages of pre-implantation embryos, from zygote to blastocyst, has been reported by Chen et al. It is interesting to note that opioid receptors are expressed both on the membrane and in the cytoplasm of pre-implantation embryos. This expression pattern suggests the involvement of opioid signalling during pre-implantation embryo development, as well as the potential detrimental effects of drug abuse on pre-implantation embryo development, implantation and pregnancy outcomes (Chen *et al.*, 2014; Kalyuzhny *et al.*, 1997). These findings suggest that the pre-implantation embryo is a direct target for the opioid system (Chen *et al.*, 2014).

In mouse models, exposure to kerack during pregnancy resulted in a significant decrease in the developmental potential of the morula stage into the blastocyst stage. The addicted group exhibited a decrease in the total number of blastocyst cells and inner cell mass, as well as an increase in apoptosis rates compared with the control group (Mohammadzadeh *et al.*, 2017). However, cocaine administration in rabbits showed no effects on the number of ovarian follicles, retrieved oocytes, IVF results or cleavage rate. Nevertheless, hormonal changes were observed in the rabbits, including an increase in follicular fluid oestradiol and a decrease in progesterone levels in both serum and follicular fluid during the periovulatory stage. The authors suggested that these hormonal changes induced by cocaine administration may affect fertility through delayed granulosa cell luteinization (Kaufmann *et al.*, 1990). When zebrafish embryos were exposed to cocaine, a very low mortality rate and no obvious abnormalities were observed. However, alterations in protein expression levels indicated detrimental effects of cocaine exposure on early embryo development (Parolini *et al.*, 2018).

In mice, intraperitoneal injection of MAMP (10 mg/kg/day) for 14 days resulted in a decrease in fertilization and cleavage rates. However, shorter-term injection of MAMP for 2 days did not impact embryo development or fertilization rates (Nezhad *et al.*, 2016).

The intraperitoneal injection of morphine into mice on days 2–3 of pregnancy disrupted the expression of opioid receptors and normal development of pre-implantation embryos into blastocysts. Additionally, normal calcium oscillation was inhibited in embryos exposed to morphine (Chen *et al.*, 2014). Interestingly, Chernov et al. reported that culturing embryos in a medium supplemented with β-endorphin improved the development of two-cell embryos into the blastocyst stage (Chernov *et al.*, 2009). The presence of mu opioid receptors has been documented in mouse oocytes and granulosa cells, with varying expression patterns depending on the stage of maturation. Furthermore, morphine has been shown to improve the development of oocytes to the blastocyst stage by modulating the PI3K/Akt and MAPK pathways (Olabarrieta *et al.*, 2019).

The abuse of marijuana can have an impact on IVF results. Women who have used marijuana more than 90 times exhibited a lower number of oocytes retrieved and embryos transferred compared with non-users ($P < 0.05$). However, mild (1–10 times) and moderate (11–90 times) marijuana use showed no significant effects on the number of oocytes retrieved. It is worth noting that even marijuana abuse by couples up to 1 year before IVF significantly decreased the number of retrieved oocytes, fertilized oocytes and embryos transferred. Furthermore, infants born to parents who abused marijuana had significantly lower birth weights (Klonoff-Cohen *et al.*, 2006). Both CB receptors (cannabinoid receptors) are expressed in 2-cell embryos. However, activation of the CB1 receptor following marijuana abuse can result in arrested pre-implantation embryo development (Paria *et al.*, 1998; Sun & Dey, 2008). Nonetheless, a cohort study comparing IVF outcomes between marijuana users and non-users found no significant differences in the number of retrieved oocytes and their maturity, fertilization rate, peak serum oestradiol, embryo quality, implantation rate and ongoing pregnancy between the two groups (Har-Gil *et al.*, 2021).

A study conducted by Favetta demonstrated that THC decreased the ability of bovine oocytes to undergo nuclear maturation, leading to reduced fertilization competence and poor embryonic development (Favetta, 2023).

According to the literature, nicotine exposure during embryonic development can impact on embryo development. A study utilizing a time-lapse system reported impaired early embryonic development in women who smoke compared with non-smoking women (Fréour *et al.*, 2013). Nicotine-treated embryos exhibited notable variations in developmental stages compared with control embryos (Kamsani *et al.*, 2010). In mice, nicotine treatment caused slower transport of the embryo through follopian tube due to reduced motility which may explain the higher incidence of ectopic pregnancies in smoking women (DiCarlantonio & Talbot, 1999). The number of hatched blastocysts also decreased at various nicotine concentrations (Kamsani *et al.*, 2013). Another study demonstrated that exposure to cigarette smoke led to a higher occurrence of multinucleated blastomeres in developed bovine blastocysts (Liu *et al.*, 2008). In a study, Banafshi and colleagues showed that smoking had a significant effect on the expression of pluripotency genes, apoptotic genes and the aryl hydrocarbon receptor (AhR) gene (Banafshi *et al.*, 2022). Collectively, these findings highlight the complex and dose-dependent effects of cigarette smoke on embryo development and gene expression during critical stages of embryogenesis.

Furthermore, studies have identified a correlation between active smoking in women and a delay in blastocyst expansion during ART. Women who smoke while undergoing ART procedures tend to experience a longer timeframe for blastocyst expansion in comparison to non-smokers (day 6 vs day 5) (Bourdon *et al.*, 2020). In another study, nicotine exhibited adverse effects on the secondary meiotic spindle structures and overall embryonic development, with the severity of the effects being dependent on the dose (Liu *et al.*, 2008). A recent study conducted in 2024, Ryoma Taniguchi et al., have examined the effects of male partners' smoking status on embryo kinetics in IVF. Despite observing abnormalities in the secondary meiotic spindle structures and impaired embryonic development *in vitro* due to nicotine exposure, the study detected no notable impact on

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Table 2. Summary of the substance's effects on sperm

Substances		Species	Results	Reference
Opioids	Opiate	Human	Decreased sperm concentration, catalase-like and superoxide dismutase-like activity. Increased the sperm DNA fragmentation.	(Safarinejad *et al.*, 2013)
	Tramadol	Mouse	Decreased sperm concentration, motility and vitality. Reduced spermatogenesis in the germinal epithelium of affected seminiferous tubules.	(Azari *et al.*, 2014)
		Human	Decreased sperm counts, sperm vitality, and free testosterone levels. Increased abnormal sperm morphology and prolactin levels.	(Bassiony *et al.*, 2020)
	Heroin	Human	Increased semen PH and sperm histone replacement abnormalities. Decreased sperm motility and viability.	(Nazmara *et al.*, 2019)
			Decreased sperm total motility and viability. Lower aminopeptidase N (APN) and endopeptidase (NEP) gene expression levels. Correlation between duration of drug dependence and sperm viability, sperm motility, and expression levels of APN gene.	(Rezaei-Mojaz *et al.*, 2020)
			Leukocytospermia Asthenozoospermia DFI elevation in sperm cells Epigenetic alteration	(Nazmara *et al.*, 2021)
			An altered cargo of small RNAs (sRNAs) in human spermatozoa.	(Gornalusse *et al.*, 2023)
	Diacetylmorphine	Human	Decreased histone-to-protamine ratio, sperm morphology, viability, and motility.	(Ebrahim *et al.*, 2020)
	Codeine	Human	Decreased motility and plasma membrane integrity of spermatozoa at any tested concentration. Increased sperm 8-OHdG level. Negative association between sperm 8OHdG level, motility, plasma membrane integrity, and DNA integrity of spermatozoa.	(Akhigbe *et al.*, 2021)
	Codeine	Rabbit	Significant reduction in sperm membrane integrity and sperm parameters (normal morphology, viability, count, and motility). Significant increase in oxidative damage, caspase 3 activity, and sperm DNA fragmentation.	(Ajayi & Akhigbe, 2020)
Cannabinoids	Marijuana	Human	Significantly higher sperm concentration in men who had ever smoked marijuana than men who had never smoked marijuana. Lower sperm concentration, total sperm motility and FSH among marijuana smokers. No association between marijuana smoking and other reproductive hormones or sperm DNA integrity markers.	(Nassan *et al.*, 2019)
			Decreased sperm concentration and total sperm count in regular marijuana smoker (more than once per week). Higher levels of testosterone in marijuana smokers.	(Gundersen *et al.*, 2015)
			Decreased sperm motility and increased abnormal morphology.	(Carroll *et al.*, 2020)
	Δ9-THC	Mouse	No significant effect on mRNA expression of CB1 in the testis. No alterations in testes histology, sperm motility or concentration, and methylation of evaluated three CpG regions of CB1 in the embryos produced via *in vitro* fertilization (IVF).	(López-Cardona *et al.*, 2018)
		Bovine	Alteration of motility, morphology, capacitation, and mitochondrial potential of spermatozoa. Disruption of the expression of key microRNAs associated with early embryonic development.	(Favetta, 2023)
Stimulant	MAMP	Rat	Decreased number of seminiferous tubules cells and spermatogenesis. Decreased number of spermatogonia, primary and secondary spermatocytes. Decreased spermatogenesis indices (mean seminiferous tubules diameter, tubular differentiation index, repopulation index, and spermiogenesis index).	(Saberi *et al.*, 2017)
			Decreased cell proliferation and the ratio of proliferation to apoptosis. Increased apoptosis in spermatogonia and primary spermatocytes. The presence of significant gaps between the layer of spermatogonia and other layers of cells.	(Alavi *et al.*, 2008)
		Mouse	Increased percentage of apoptotic seminiferous tubules. Decreased serum testosterone level and inhibited male copulatory behaviour	(Yamamoto *et al.*, 2002)
Hallucinogens	MDMA (ecstasy)	Rat	Decreased Gonadotropin-releasing hormone (GnRH) level and serum testosterone	(Dickerson *et al.*, 2008)

(*Continued*)

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Table 2. (*Continued*)

Substances		Species	Results	Reference
Nicotine	Nicotine	Human	Decrease in semen volume, sperm concentration, and total sperm count	(Asare-Anane *et al.*, 2016)
			Significant link between smoking and decreased sperm concentration	(Künzle *et al.*, 2003)
			decreased levels of zinc and Ca+2 ATPase in seminal plasma - reduced sperm motility	(Kumosani *et al.*, 2008)
			- Alterations in axonemal microtubules and tails - Detrimental impact on sperm motility	(Yeung *et al.*, 2009)
			Damage of acrosome reaction	(Zalata *et al.*, 2004)
			Damage of capacitation	(Shrivastava *et al.*, 2014)
			Increased chromosomal segregation anomalies	(Pereira *et al.*, 2014)
			Increased germline mutations	(Linschooten *et al.*, 2013)
			- Increased levels of ROS production - Increased sperm DNA fragmentation index - Increased levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) in semen samples	(Kumar *et al.*, 2015)
			- Decreased activity of sperm glutathione peroxidase (GPx-1, 4) - Decreased mRNA expression of glutathione reductase in spermatozoa	(Viloria *et al.*, 2010)
			Alteration of gene expression at the mRNA and miRNA levels in the spermatozoa	(Marchetti *et al.*, 2011)
			Increased abnormal number of autosomal chromosomes	(Beal *et al.*, 2017)
			Alterations in sperm protamination	(Hamad *et al.*, 2014
			Alterations in the miRNA content of spermatozoa	(Marczylo *et al.*, 2012)
		Mouse	- Induction of apoptosis in Leydig cells - Inhibition of androgen biosynthesis	(Kim *et al.*, 2005)
			Increased apoptosis in testes	(La Maestra *et al.*, 2015)
			Alterations in sperm protein expression related to energy metabolism, reproduction, and the development of structural molecules	(Chen *et al.*, 2015)
		Rat	Reduction of the number of germ cells, Leydig cells, and Sertoli cells	(Ahmadnia *et al.*, 2007)
			irregular and thickened basal lamina of seminiferous tubules	(Abdul-Ghani *et al.*, 2014)
			- Decreased testicular gametogenesis and steroidogenesis - Decreased steroidogenic acute regulatory protein expression	(Jana *et al.*, 2010)
Alcohol	Alcohol	Mouse	Alterations in none-coding small RNAs in sperm cells	(Rompala *et al.*, 2018)
			Changes in non-coding RNAs in sperm cells associated with foetal growth restriction in the offspring	(Bedi *et al.*, 2019)
		Rat	Increased DNA strand breaks	(Kotova *et al.*, 2013)
		Human	- Negative impact on sperm concentration, total sperm count and normal sperm morphology - Alterations in reproductive hormones	(Jensen *et al.*, 2014)
			- No negative impact on semen quality - Increased percentage of microcephalic sperms	(Lwow *et al.*, 2017)

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Table 3. Summary of the substance's effects on pre-implantation embryos

Substances		Species	Results	Reference
Opioids	β-endorphin	Mouse	Improvement of the two-cell embryo development into blastocyst stage after culture of embryos in medium supplemented with β- endorphin.	(Chernov *et al.*, 2009)
	Morphine	Mouse	Disturbed expression of opioid receptors and normal development of pre-implantation embryos into blastocyst. Inhabitation of normal calcium oscillation in embryos.	(Chen *et al.*, 2014)
Cannabinoids	Marijuana	Human	Fewer oocyte retrieval and embryo transfer in woman with heavy marijuana use.	(Klonoff-Cohen *et al.*, 2006)
			No significant difference regarding the number of retrieved oocytes, maturity of oocytes, fertilization rate, peak serum oestradiol, and embryo quality and implantation rate between marijuana users and none-users.	(Har-Gil *et al.*, 2021)
	Δ9-THC	Bovine	Decreased ability of oocytes to nuclear maturation, leading to reduced fertilization competence and poor embryonic development.	(Favetta, 2023)
Stimulants	Kerack	Mouse	Decreased developmental potential of morulla into blastocyst stage. Decreased total number of blastocyst cells and inner cell mass. Increased apoptosis rate of blastomer cells.	(Mohammadzadeh *et al.*, 2017)
	Cocaine	Rabbit	No differences in the number of follicles present and oocytes retrieved, or rates of IVF and cleavage. Induction of hormonal disturbances.	(Kaufmann *et al.*, 1990)
		Zebrafish	Very low mortality rate and no obvious abnormality in embryos. Alteration of the proteome of embryos.	(Parolini *et al.*, 2018)
	MAMP	Mouse	Increased number of fragmented oocytes. Reduction of fertilization and cleavage rate.	(Nezhad *et al.*, 2016)
		Rat	Decreased yolk sac diameter, the crown-rump length and the somite number. Increased number of embryos with abnormalities such as microcephaly, neural tube defects, incomplete rotation of the body axis, and tortuous spinal cord.	(Yamamoto *et al.*, 1995)
Nicotine	Nicotine	Human	Impaired early embryonic development	(Fréour *et al.*, 2013)
			Delay in blastocyst expansion following ART	(Bourdon *et al.*, 2020)
		Mouse	- Reduced number of retrieved embryos - Decreased developmental capacity of the embryos - No blastocyst formation - Increased ROS production	(Kamsani *et al.*, 2010)
			Decreased number of hatched blastocysts at various nicotine concentrations	(Kamsani *et al.*, 2013)
			- Decreased embryo development - Significant effect on the expression of pluripotency genes, apoptotic genes, and the aryl hydrocarbon receptor (AhR) gene	(Banafshi *et al.*, 2022)
		Bovine	- No impact on cleavage and blastocyst rates in low doses - Decreased cleavage rates, increased embryo development arrest in high doses - Impaired alignment or segregation of chromosomes and the formation of abnormal nuclear structures - Lower cell numbers in blastocysts - Higher occurrence of multinucleated blastomeres	(Liu *et al.*, 2008)
Alcohol	Alcohol	Human	decreased proliferation of trophoblast cells	(Lui *et al.*, 2014)
		Rat	Detrimental effect on pre-implantation embryo development	(Sandor *et al.*, 1981)
		Mouse	- Delayed embryo development - Morphological abnormality in embryo - Impaired blastocyst hatching - Embryo loss through fragmentation due to alterations induced in the oocyte.	(Cebral *et al.*, 2000)
			No negative effects on embryo development	(Wiebold & Becker, 1987)
			Impaired levels of H3K9 acetylation in the pre-implantation embryos	(Fang *et al.*, 2015)
			- Severe growth retardation placentae and embryos - No effect on DNA methylation at maternal and paternal alleles of embyos - Less methylation in the paternal alleles of ethanol-treated placentae	(Haycock & Ramsay, 2009)

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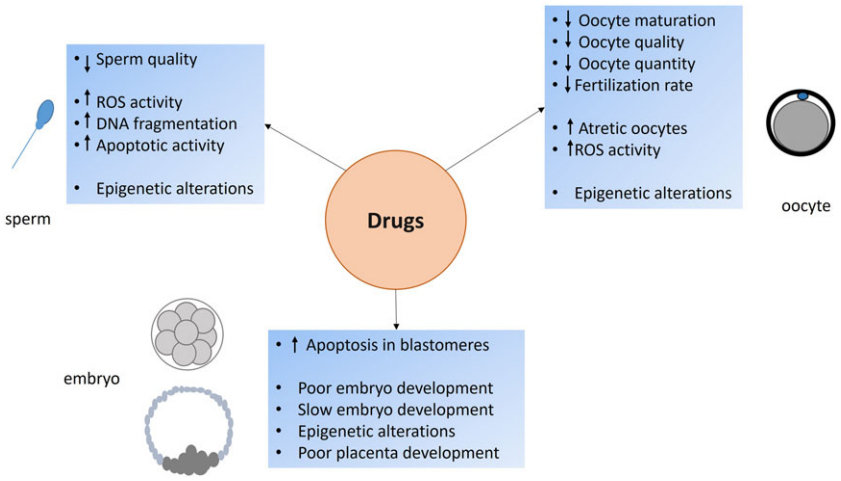

Figure 1. Detrimental effects of drugs on pre-implantation embryos, sperm and oocyte based on different studies.

blastocyst formation time, morphology of embryos or clinical outcomes (Taniguchi *et al.*, 2024). Similarly, another study indicated that male tobacco smoking did not have significant effects on early embryo morphology or kinetics (Frappier *et al.*, 2022).

Although it has been evidenced that chronic alcohol consumption during pregnancy has harmful effects on the foetus (Wilhoit *et al.*, 2017), the effects of alcohol consumption on pre-implantation embryo development, implantation or uterus receptivity is unclear. Studies on alcohol consumption and human embryo implantation are scarce, but *in vitro* studies suggested that alcohol may exert harmful effects on human placental cells and also granulosa cells (Ahluwalia *et al.*, 1992; de Angelis *et al.*, 2020; Wimalasena *et al.*, 1993). The presence of ethanol in the oviduct and uterine lumen of female rats following chronic alcoholization may affect pre-implantation embryo development (Sandor *et al.*, 1981). Cebral et al. demonstrated that preconceptional chronic ethanol ingestion by prepubertal female mice can lead to retarded embryo development, morphological abnormality in embryo, impaired blastocyst hatching and embryo loss through fragmentation due to alterations induced in the oocyte (Cebral *et al.*, 2000). Wiebold et al. showed that alcohol ingestion during the first 3 days of pregnancy had no effect on embryo development (Wiebold & Becker, 1987). Lui et al. investigated the effect of ethanol and acetaldehyde on the first trimester human placental cell and showed its detrimental effects on proliferation of trophoblast cells (Lui *et al.*, 2014).

Early prenatal alcohol exposure can change epigenetic marks as well as impacting cell differentiation, embryo development and the adult phenotype (Wallén *et al.*, 2021). According to the mouse model, alcohol ingestion by females can influence the levels of H3K9 acetylation in pre-implantation embryos (Fang *et al.*, 2015). Haycock et al. evaluated the effect of ethanol exposure during pre-implantation development of mouse embryos on DNA methylation at the H19 imprinting control region (ICR). Although severe growth retardation was observed in ethanol-exposed placentae and embryos, DNA methylation at maternal and paternal alleles was not affected in embryos. However, less methylation was observed in paternal alleles of ethanol-treated placentae (Haycock & Ramsay, 2009). Rao et al. evaluated the correlation between alcohol consumption and ART outcomes in a meta-analysis study. The findings showed that maternal alcohol consumption was negatively related to pregnancy after IVF/ICSI treatment (Nicolau *et al.*, 2014). Much more studies are necessary to fully understand the association between alcohol consumption and embryo implantation.

However, there is limited data available on the effects of different addictive drugs on pre-implantation embryos and IVF/ICSI outcomes, further research is essential in this field, especially at the molecular level. Table 3 illustrates the effects of substances on pre-implantation embryos based on different studies.

Conclusion

The existing evidence suggests that drug abuse can have negative effects on oocytes, sperm cells and pre-implantation embryos, which can negatively affect fertility. Numerous studies have indicated that drug abuse can lead to subfertility or infertility by affecting gametes and reproductive processes. Figure 1 briefly explains detrimental effects of drug abuse on pre-implantation embryos and gametes based on different studies. It has been evidenced that drug consumption in men can impair fertility by affecting semen quality, DNA integrity, antioxidant activity and hormonal disturbance. Impaired protamination, altered expression of genes and changes in non-coding RNAs are epigenetic alterations following drug abuse that can have detrimental effects on offspring health. In women, similar to men, drug abuse can lead to oxidative stress and epigenetic changes, which can contribute to reduced fertility in these individuals. Furthermore, substance exposure can disrupt ovarian follicular development and oocyte maturation, leading to lower quality and quantity of oocytes. Decreased ovarian reserve following drug abuse can be due to hormonal imbalances and impaired folliculogenesis. As mentioned, both oocytes and sperms, when exposed to drugs, can have detrimental impact on embryos and fertility. Pre-implantation embryos can also be exposed to drugs in addicted women. According to the literature, exposure to drugs before implantation can lead to poor and slow embryo development, increased apoptosis, decreased cell count, epigenetic changes in both embryo and placenta as well as poor placenta development which can subsequently lead to complications for pregnancy and foetus. However, it is important to acknowledge that there is still a significant gap in the current literature when it comes to understanding the effects of different drugs on the capability and fertilization potential of gametes especially oocytes, as well as pre-implantation embryos. More comprehensive studies, particularly at the molecular level, are needed in order to gather further insights into these complex mechanisms. Additionally, it is

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important to note that the available data in this field are not yet conclusive, and there are several confounding variables that need to be considered in human studies. Therefore, the conduction of large-scale clinical trials is strongly recommended in order to provide more definitive conclusions. By emphasizing the gaps in the current knowledge and the need for further research, the conclusion highlights the importance of continuing to explore the effects of drugs on fertility and reproductive processes.

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